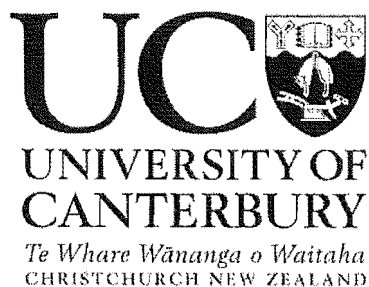


CROSS METATHESIS AND RING-CLOSING METATHESIS REACTIONS OF MODIFIED AMINO ACIDS AND PEPTIDES

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ABSTRACT

This thesis investigates the application of cross metathesis and ring-closing metathesis to amino acid and peptide-based substrates that are suitably modified to contain an olefin tether.

Chapter One introduces olefin metathesis, describes the mechanism of cross metathesis (CM) and ring-closing metathesis (RCM), and outlines the catalysts that can be used for these transformations. The application of CM and RCM to amino acid and peptide-based systems is reviewed.

Chapter Two describes the CM coupling between modified lysine- (**2.34** – **2.37**, **2.43**), serine- (**2.45**, **2.46**), and cysteine-based (**2.48**, **2.49a**, **2.51**) amino acids and dipeptides (**2.54**, **2.57**) to a terminal alkene (**2.61**, **2.65**), carbohydrate (**1.51b**), or fatty acid (**2.76**) target compound using catalyst **1.17**. The amino acid and dipeptide-based CM substrates were prepared by side-chain acylation of the parent amino acid with carboxylic acids containing variable but controllable olefin tether lengths. A CM model study in which these amino acid-based substrates were coupled to terminal alkene **2.61** and **2.65** gave CM products **2.66** – **2.74**. CM was then carried out between amino acid-based substrates and a carbohydrate (**1.51b**) or fatty acid derivative (**2.76**), that afforded a novel series of glycoamino acids (**2.80** – **2.85**) and lipoamino acids (**2.94** – **2.101**).

Chapter Three describes the synthesis of amino acid dimers by CM. Two serine-based (**3.22** – **3.23**) and two cysteine-based (**3.24** – **3.25**) symmetrical dimers along with two unsymmetrical serine-cysteine dimers (**3.26** – **3.27**) were prepared from the same side-chain acylated amino acid substrates described in chapter 2. These compounds are examples of novel cross-linked amino acid-based dimers, and further illustrate the versatility of the CM methodology developed in this thesis.

Chapter Four describes the synthesis of cyclic amino acids and dipeptides via RCM of acyclic precursors that are suitably modified with acyl olefin tethers of variable length. Cyclic compounds based on lysine (**4.6**, **4.13**), serine (**4.31**, **4.33**), and cysteine (**4.40**, **4.42**)

single amino acid residues, and compounds based on lysine (**4.16**, **4.21**, **4.27**), serine (**4.37**), and cysteine (**4.45**, **4.46**) dipeptides were prepared. All these compounds were constructed using the same, versatile general method, which involves acylation of the natural amino acid substrate with a carboxylic acid of controllable olefin tether length followed by RCM with catalyst **1.17** to give cyclic products containing variable ring sizes.

ABBREVIATIONS

BOP-Cl	benzotriazol-1-yloxy-tris(dimethylamino) phosphonium hexafluoro-phosphate
Boc	<i>tert</i> -butoxycarbonyl
br s	broad singlet (in NMR)
Cbz	carbobenzyloxy
CM	cross metathesis
COSY	correlation spectroscopy
d	doublet (in NMR)
DCM	dichloromethane
DIPEA	<i>N,N</i> -diisopropylethylamine
DMAP	4-(dimethylamino)pyridine
DMF	<i>N,N</i> -dimethylformamide
DMSO	dimethylsulfoxide
EDCI	1-[3-(dimethylamino)propyl]-3-carbodiimide hydrochloride
Fmoc	fluorenylmethoxy carbonyl
h	hour(s)
HATU	<i>O</i> -(7-azabenzotriazol-1-yl)- <i>N,N,N',N'</i> -tetramethyluronium hexafluoro-phosphate
HOBT	1-hydroxybenzotriazole
HPLC	high performance liquid chromatography
HRMS	high resolution mass spectrometry
Hz	hertz (in NMR)
<i>J</i>	coupling constant (in NMR)
m	multiplet (in NMR)
MD	molecular dynamics
min	minute (s)
mp	melting point
NMR	nuclear magnetic resonance
ppm	parts per million
q	quartet (in NMR)

RCM	ring closing metathesis
rt	room temperature
s	singlet (in NMR)
t	triplet (in NMR)
TCE	1,1,2-trichloroethane
TEA	triethylamine
TFA	trifluoroacetic acid
THF	tetrahydrofuran
TLC	thin-layer chromatography
SPPS	solid-phase peptide synthesis

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CHAPTER ONE

INTRODUCTION

1.1 METATHESIS

Over the past decade, olefin metathesis has been firmly established as an important and general technique in synthetic organic chemistry. It is a carbon-carbon bond forming reaction in which a transition metal catalyses the exchange of alkylidene groups of two independent olefins to give a newly substituted olefinic product. The word “metathesis” comes from the Greek *μεταθεσις* (metathesis), meaning transposition. The first reports of olefin metathesis reactions were in the 1950’s when researchers at Du Pont, Standard Oil and Philips Petroleum reported that propene reacted to give ethylene and 2-butenes when heated with $[\text{Mo}(\text{CO})_6]$ on alumina. Since that time the mechanistic studies of Chauvin and metathesis catalyst development by the Schrock and Grubbs research groups have propelled the reaction of olefin metathesis into the limelight and into the fume hoods of organic chemist’s the world over.

Olefin metathesis provides an alternative to more traditional methods for carbon-carbon bond formation such as the Heck and other palladium-mediated reactions. There are a number of different types of metathesis reactions, as summarised in Figure 1.1. Cross metathesis (CM) reactions can be carried out on either alkenes¹ (equation 1a) or alkynes² (equation 1b), likewise for ring-closing metathesis reactions (RCM) (equation 2a)³ and (equation 2b).² This thesis describes alkene CM and RCM reactions carried out with modified amino acid and peptide-based substrates.

Other types of metathesis reactions include acyclic diene metathesis polymerization (ADMEP)⁴ (equation 3) and ring-opening metathesis polymerization (ROMP)⁵ (equation 4), which are increasingly finding application in polymer chemistry. Enyne metathesis (EYN)⁶ (equation 5) products are intrinsically set up for cycloaddition reactions on the resulting diene, for example Diels Alder reactions, which can be used to further derivatise molecules. Ring-opening cross metathesis (ROCM)⁷ (equation 6) has also been used in synthetic chemistry. In addition to discrete, one step metathesis reactions (equations 1-6), there are several variations of one pot reactions that encompass more than one metathesis transformation. Some examples of these are the tandem RCM/CM⁸ (equation 7) and domino metathesis reactions⁹ (equation 8). As only alkene CM and RCM reactions are

carried out in this thesis, the other types of metathesis reactions will not be further discussed.

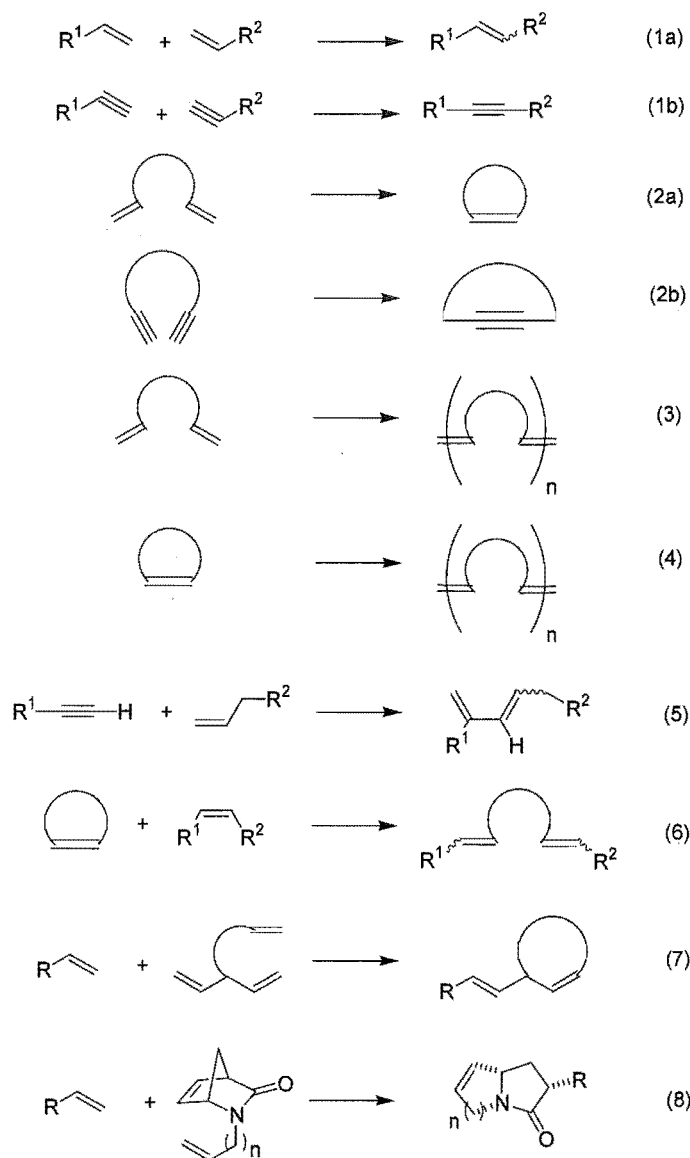


Figure 1.1. Olefin metathesis reactions

1.2 MECHANISM

The generation of a metallacyclobutane intermediate by alternating [2+2] cycloadditions and cycloreversions is the generally accepted mechanism for alkene metathesis. This mechanism, originally proposed by Chauvin in 1971,¹⁰ has been confirmed over the years with various mechanistic and labeling studies¹¹⁻¹⁴ and complex mechanistic elucidation.^{15,16}

A simplistic version of the “Chauvin carbene mechanism” is shown in Figure 1.2. The catalytic cycle begins with the dissociation of a ligand (Y) from the ruthenium (step **A**) to form the active, 14-electron catalytic species. In the case of Grubbs phosphine catalyst **1.17** (see section 1.3) Y is the tricyclohexylphosphine (PCy₃) ligand, while for the phosphine free catalyst **1.20** the dissociating ligand Y is *i*PrO. Co-ordination of an alkene substrate to the 14-electron species (step **B**) can then occur, which is in competition with the re-association of Y (reverse of step **A**). Depending on electronic and steric effects, the co-ordination of substrate H₂C=R¹ (Step **B**) is favoured over both re-coordination of ligand Y and co-ordination of the other alkene substrate H₂C=R².^{2,13,14}

The [2+2] cycloaddition of alkene H₂C=CHR¹ to the 14-electron Ru species (step **B**) results in the formation of a four-membered metalla-cycle. This “ruthenacycle” then fragments rapidly (step **C**) to give a newly substituted alkylidene with the release of XHC=CH₂. X is the functional group on the catalyst carbene in the first catalytic cycle, for example the phenyl group in catalyst **1.17**. In subsequent catalytic cycles X is the functional group of the olefin XHC=CHR², for example X = H in terminal alkenes which gives ethylene as the alkene byproduct (Step **C**). When this occurs the reaction is thermodynamically driven by the removal of the volatile ethylene gas from the reaction mixture.

A second alkene then reacts regio- and stereoselectively with alkylidene [Ru]=CHR¹ to give a second metallacyclobutane (steps **D**¹ or **D**²). The formation of the metalla-cycle shown in step **D**¹ is highly favoured over that in **D**² (known as degenerate metathesis).¹⁷ The favoured metallo-cycle resulting from step **D**¹ then fragments to regenerate the active 14-electron catalytic species and the desired CM product R¹HC=CHR² (step **E**).

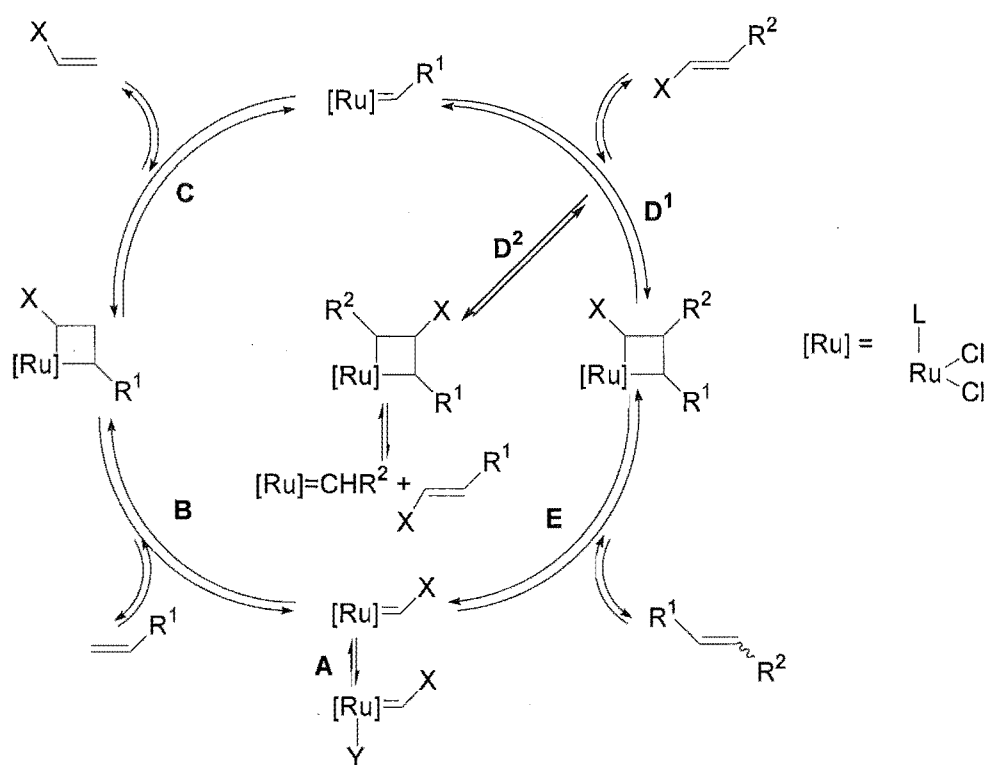


Figure 1.2. Catalytic cycle of olefin metathesis

1.3 METATHESIS CATALYSTS

Numerous catalysts have been reported that facilitate olefin metathesis (see Figures 1.3 and 1.4). Over the past few decades the development of catalysts with improved reactivity, selectivity, and stability has enabled the application of olefin metathesis to a wide range of synthetic and organic chemistry problems. Reviews have been written summarising the history of olefin metathesis catalyst development over the years.^{3,18,19} The following section summarises key aspects of the structure, functional group tolerance, and development of metathesis catalysts.

Schrock's molybdenum alkylidene catalyst **1.1** (Figure 1.3) was one of the first metathesis catalysts to be developed.^{20,21} This catalyst is known to tolerate substrates with β -lactam backbone²² and acrylonitrile functionalities, but substrates with functional groups such as unprotected amines, free alcohols, acetate groups, enones, and enoic esters are not compatible. Despite these drawbacks, commercially available **1.1** is highly reactive towards a range of substrates that contain a variety of functional groups. In general, Schrock's catalyst **1.1** gives shorter reaction times and higher yields than the more widely used ruthenium catalysts such as **1.10**. However, its general use remains somewhat problematic due to sensitivity towards atmospheric oxygen.

There are a number of other molybdenum catalysts available (see Figure 1.3), for example chiral carbenes such as **1.2**,²³ **1.3**,²⁴ and **1.4**,²⁵ the latter of which is activated in DCM by the formation of an alkylidyne species in situ. Catalyst **1.4** has found use in alkyne cross-metathesis reactions that contain both electron donating and electron withdrawing substituents, and where tolerance to polar groups such as ethers, esters, nitriles, acetals, sulfones, and silyl ethers is required. Hoveyda and Schrock have more recently developed a series of chiral solid-supported molybdenum complexes, for example catalyst **1.5**, that are efficient in enantioselective olefin metathesis reactions.²⁶

Titanium and tungsten metals have also been used as the basis for metathesis catalysts (Figure 1.3). For example the titanium carbene **1.6**²⁷ and the tungsten catalysts **1.7** and **1.8** have found use in CM reactions.^{10,28}

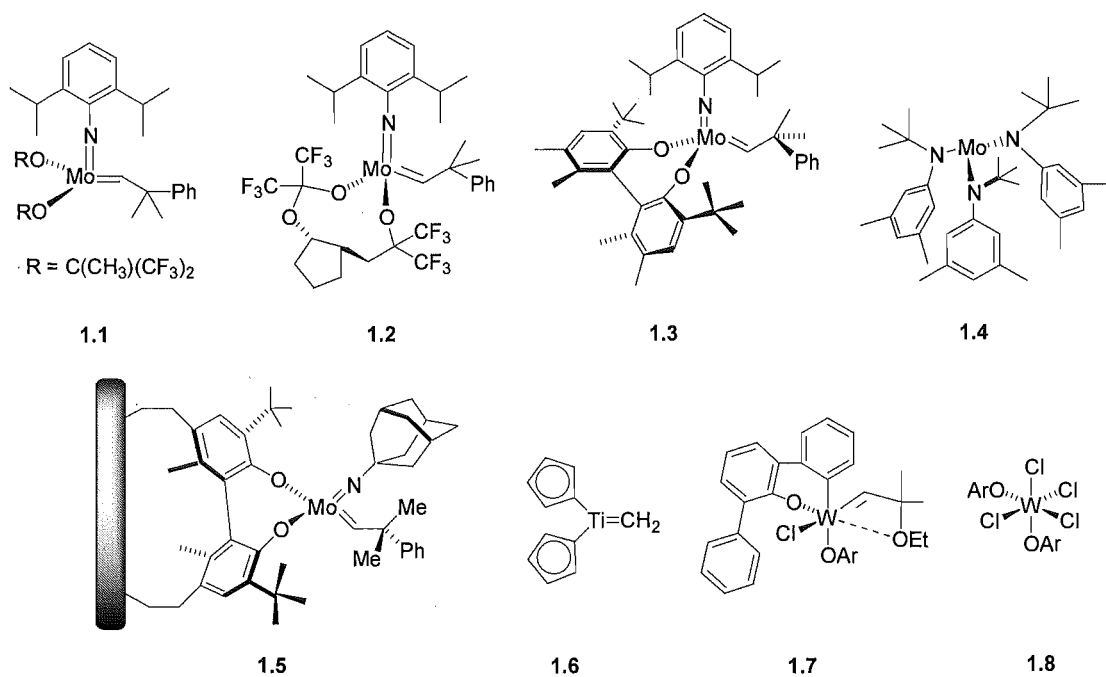


Figure 1.3. Molybdenum, Titanium, and Tungsten olefin metathesis catalysts

Catalyst **1.9** (Figure **1.4**), one of the first ruthenium carbene catalysts reported,²⁹ has found wide use in RCM reactions involving conformationally constrained amino acids and peptides.³⁰ However its use has been superseded by what remains the most commonly used olefin metathesis catalyst - Grubbs “first generation”^{*} **1.10**, a versatile and reliable tool for organic syntheses.³¹ Commercially available **1.10** initiates metathesis reactions more rapidly than catalyst **1.9**, and can tolerate functionalities such as carbamate hydrogens,³² unprotected carboxylic acids,³³ and a wide range of peptide protecting groups,³² while remaining relatively air and moisture stable. Despite its widespread applications in metathesis reactions, catalyst **1.10** does exhibit some drawbacks such as sluggish reactivity, especially towards unprotected homoallylic alcohols, allyltrimethylsilane,³⁴ and substituted double bonds.³⁵ The bi-metallic catalyst **1.11** was reported to have similar catalytic activity to **1.10** but with the advantage of increased stability, ease of storage and an ability to be recovered and recycled.³⁶

“First generation” water-soluble ruthenium catalysts such as **1.12** and **1.13** have been developed³⁷ to allow metathesis reactions to be carried out in polar solvents, a feature that is particularly important if biological applications involving water soluble substrates are to be fully realised. These catalysts, synthesized by a ligand exchange of catalyst **1.10**, show good RCM activity in solvents such as water, methanol, water/THF mixtures, and also benzene.³⁷ Catalysts **1.12** and **1.13** have, however, found limited use to date due to their high air sensitivity in solution, decomposing rapidly to form a bright green solution in the presence of trace levels of oxygen. It is interesting to note that while catalyst **1.13** is also soluble in DCM it does not show activity in this solvent due to instability.³⁸ A number of other “first generation” ruthenium based catalysts have also been developed including the infrequently used photo inducible dimer **1.14**³⁹ and the chiral benzimidazolyidene catalyst **1.15** that has comparable activity to **1.10**.⁴⁰

Hoveyda reported a “first generation recoverable” ruthenium-based complex **1.16** in 1997.⁴¹ Compared to **1.10**, this catalyst has the advantage that it can be purified for re-use from reaction mixtures in high yield by silica gel-based chromatography.¹² However, in general while **1.16** shows similar catalytic activity to **1.10**, it is only reactive towards terminal alkenes.

^{*} In this context, a “first generation catalyst” refers to a ruthenium catalyst that contains at least one phosphine ligand, for example PCy₃, and no *N*-heterocyclic ligand.

The emergence in 1999 of the now commercially available Grubbs “second generation”^{*} catalyst **1.17** proved to be a particularly significance advance in metathesis chemistry.⁴² The steric bulk and increased σ -donor ability of the five-membered *N*-heterocyclic carbene ligand imparts improved stability and activity compared to the first generation catalyst **1.10**.⁴³ Catalyst **1.17**, which has a similar if not improved functional group tolerance compared to **1.10**, has been used to prepare tri- and tetra- substituted double bonds by both RCM and CM.⁴⁴ It has also found use in CM reactions involving α,β -unsaturated esters, ketones, and aldehydes. However, **1.17** remains unreactive towards acrylonitriles and other electron deficient substrates such as vinyl sulfones.⁴⁵ Catalysts such as **1.18** have been developed to directly compare the effect the *N*-heterocyclic ligand has on catalytic activity. In this case the bulky 6-membered ligand catalyst **1.18** was shown to have lower reactivity compared to parent **1.17**.⁴⁶ Another variant on catalyst **1.17** has been made which bears a four-membered *N*-heterocyclic carbene ligand.⁴⁷ This catalyst has been shown to have slower olefin metathesis rates as compared to **1.17**. However further studies are required into the coordination properties of this four-membered ring to better understand this difference in activity.

Water-soluble catalysts based on the “second generation” Grubbs scaffold have also been developed. The poly(ethylene glycol) conjugated catalyst **1.19** shows activity in ring-opening metathesis polymerization in water and methanol, while is completely insoluble in diethyl ether.⁴⁸ This latter property allows ease of product purification by precipitating the catalyst from the reaction mixture following metathesis. This has some advantages since homogenous metathesis catalysts are often difficult to separate from organic products.

Commercially available “second generation recoverable” phosphine-free catalyst **1.20** has been developed by Hoveyda and co-workers.⁴⁹ This catalyst is highly reactive in metathesis reactions, recyclable via silica gel-based chromatography, and extremely stable when exposed to water and/or air. This catalyst is also very effective for CM reactions where one olefin is electron deficient. For example, highly selective CM of α,β -unsaturated nitriles and acrylonitriles has been achieved using **1.20**, but not **1.17**. Catalyst **1.20** is also compatible with unprotected alcohol⁵⁰ and acid⁴³ functional groups in the metathesis substrates, and it has been used to synthesize unsymmetrical functionalised

^{*} In this context a “second generation catalyst” refers to a catalyst containing 1 or less phosphine ligands and a *N*-heterocyclic ligand.

disubstituted olefins with good stereoselectivity under mild conditions.⁵⁰ A variety of functionalities are tolerated including base-sensitive groups, and as such CM can be used to replace Wittig or Horner-Wittig reactions where substrates are base-sensitive. Catalyst **1.20** is reasonably soluble in methanol at room temperature and readily soluble at 50°C, however it remains completely inactive in water-based solvent systems.^{51,52} The dendrimeric **1.21** has been reported as an alternative to **1.20**, with an improved ability to be recycled by silica gel filtration.⁴⁹

Blechert and co-workers reported binol-based ruthenium alkylidene catalysts **1.22** and **1.23** as an addition to the growing list of second generation catalysts.^{53,54} These catalysts are significant in that they display increased activity, relative to **1.17** and **1.20**, while retaining stability even after exposure to air for one week. The improved reactivity and stability of these catalysts has been attributed to the increased steric bulk of the binol ligands.

Various ligand substitutions have been made based around catalyst **1.20**, for example Buchmeiser and co-workers⁵⁵ have synthesised ruthenium-based catalysts **1.24** and **1.25**, which have both been shown to be of equal or better activity than other ruthenium-based metathesis catalysts such as **1.17**. The two chloro ligands of **1.20** have been substituted with the even more electron withdrawing trifluoroacetate ligands in **1.25**, leading to even higher catalyst activity in some types of olefin metathesis reactions. Catalysts such as **1.24** and **1.25** possess a nitro functionality that enhances catalytic reactivity presumably due to the electron-withdrawing effect of the nitro group. This decreases the electron density at the oxygen atom of the ligating *i*PrO group, such that the dissociation of the oxygen atom to form the 14-electron catalytic species is more favoured (step A, Figure 1.2).⁵⁶

A variety of solid-phase catalysts derived from **1.20** have also been reported, including **1.26**, **1.27** and **1.28**. The deep green resin **1.27**, synthesized from **1.17** and immobilized onto a Wang resin, is a particularly stable and recyclable catalyst that shows good CM activity even with highly electron deficient olefins.⁵⁷ Solid-supported **1.26** shows high RCM activity, but much lower CM activity compared to **1.27**. Catalyst **1.28** has been used for CM reactions in methanol/water mixtures in an air atmosphere. Buchmeiser *et al* have reported catalysts immobilized onto polymeric monolithic discs that are based on the scaffold of **1.20**, that can be used in combinatorial chemistry, parallel synthesis, and high throughput screening.⁵⁸ The products of metathesis reactions carried out on solid-supports

have been shown to contain very low, residual ruthenium levels,⁵⁵ which is especially advantageous for the synthesis of useful pharmaceutical compounds. The application of CM and RCM as applied to combinatorial and parallel synthesis has been reviewed.⁵⁹ The increasing demand for solid-supported olefin metathesis catalysts is due to issues such as contamination, cost, and application to high-throughput screening programs.

More recently light fluororous versions of catalyst **1.20** have been developed by Curran and co-workers. For example catalyst **1.29**, which bears a fluororous tag, shows comparable reactivity in similar conditions to that of **1.20**.⁶⁰ This catalyst can be separated from the metathesis reaction mixture by fluororous solid-phase extraction or by filtration when added in supported form on fluororous silica gel. Catalyst **1.29** has also been shown to be recyclable and able to be reused five or more times.

Piers and colleges reported in 2004 the ruthenium-based metathesis catalyst **1.30** that has comparable catalytic activity to the Schrock family of catalysts but retains the favourable functional-group tolerance of a ruthenium-based system.⁶¹ Catalyst **1.30** is a four-coordinate, 14-electron phosphonium alkylidene that is synthesised by the transfer of the trialkylphosphine ligand onto the carbide atom using $[\text{H}(\text{OEt}_2)_2]^+[\text{B}(\text{C}_6\text{F}_5)_4]^-$. The increased activity of **1.30** relative to **1.17** is due to the lower energy barrier required for olefin binding since ligand dissociation is not required to form the catalytically active 14-electron species (step A, Figure 1.2). Catalyst **1.30** has been shown to be stable to ambient moisture and oxygen, and olefin-metathesis reactions with it have been shown to be high yielding at both refluxing and 0°C temperatures.

Ruthenium is the preferred metal centre for olefin metathesis catalysts. This is predominantly due to the exceptional functional group tolerance, rather than catalytic reactivity of ruthenium-based catalysts. A study of the reactivity of various mono-metallic catalytic complexes towards different functional groups has been carried out.⁶² This study revealed that titanium and tungsten complexes react with groups such as acids, alcohols, water, aldehydes, ketones, and amides all in preference to olefins. However, molybdenum complexes are more reactive towards olefins but still react with polar or protic groups in preference. Ruthenium complexes react preferentially with carbon-carbon double bonds over most other species, so have excellent functional group tolerance in metathesis reactions.

The continued development of olefin metathesis catalysts has made it evident that some have a higher catalytic activity in certain types of metathesis reactions, for example CM, RCM, ROMP, or EYN. A number of catalysts are now available that have different catalytic activities specifically designed towards different types of alkenes, for example tetra-substituted double bonds vs. unsubstituted, or electron withdrawing vs. donating alkene substrates. Eventually catalysts could be matched to specific metathesis reactions, to allow for good functional group tolerance, desired reaction temperature and solvent, and to give quantitative yields of easily purified products every time.

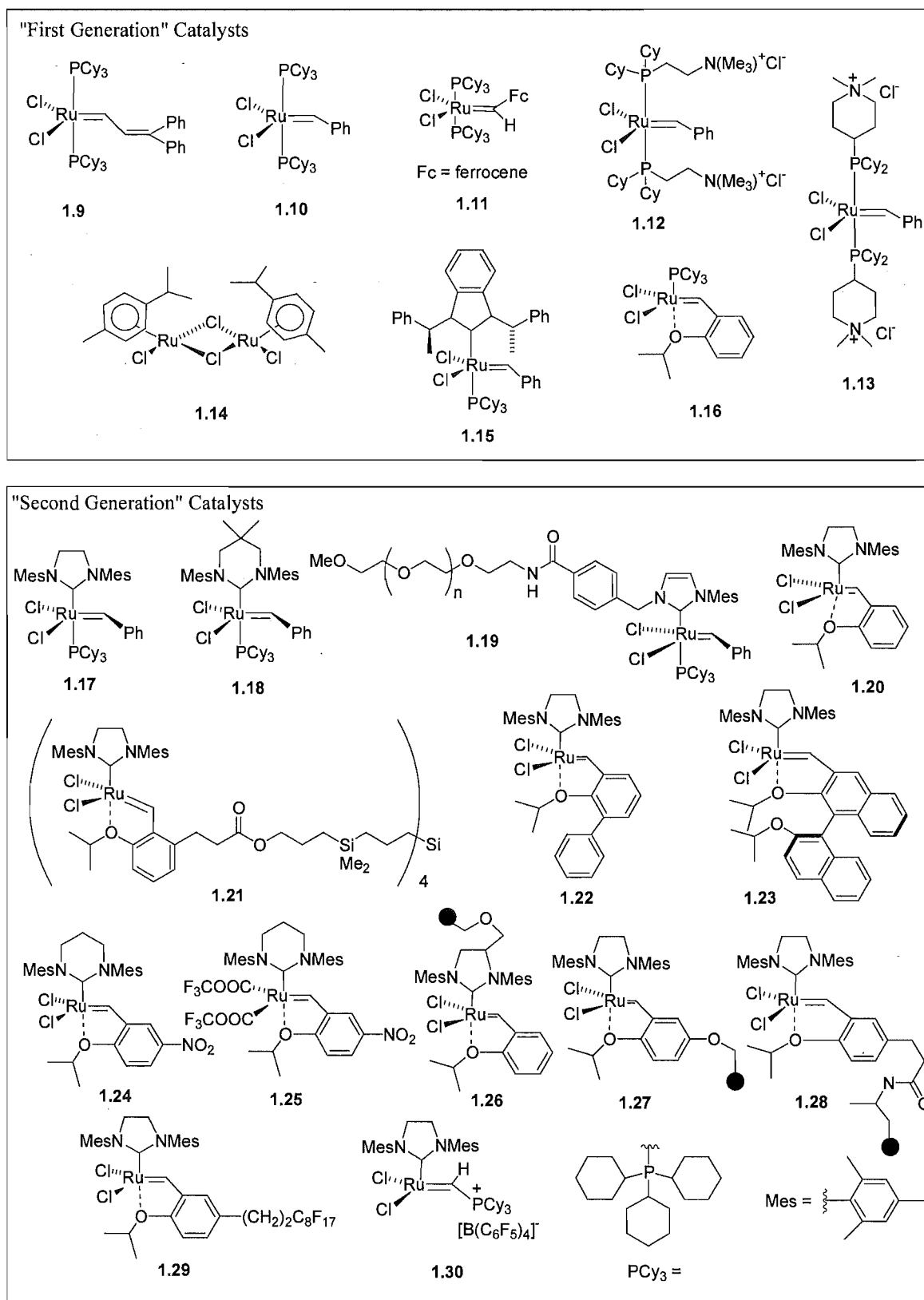


Figure 1.4. Ruthenium-based olefin metathesis catalysts

1.4 REACTION SCOPE AND CONDITIONS

While a variety of conditions have been employed in metathesis chemistry there are some standard techniques that tend to optimize reaction yields. These are based on factors that are known to influence the outcome of metathesis reactions as discussed in this section.

The efficient formation of coupled products in metathesis reactions can be facilitated by the removal of the olefinic byproduct when this is a volatile compound. For example, the conversion of vinylsilanes into CM products is quantitative when ethylene gas is removed (using a gentle stream of an inert gas). Yields can decrease considerably to around 20% in the absence of purging.¹³ A static vacuum can also be used to remove the ethylene gas when metathesis is carried out with low viscosity oils in solvent free conditions.⁶³

The most commonly utilized catalysts **1.10** or **1.17** have predominantly been used in either DCM, carbon tetrachloride, benzene, or 1,2-dichloroethane, at both reflux and at room temperature. It is important to note that catalyst **1.17** is more thermally stable than catalyst **1.10**, and as such reactions at reflux are best carried out using this catalyst. The use of reflux over room temperature conditions has the added advantage of making byproducts such as ethylene more volatile, thus thermodynamically driving the catalytic cycle. The yield of a particular cross-coupled metathesis product can often be influenced by the number of equivalents of alkene substrates used. For example yields of products in CM can be improved by increasing the number of equivalents of one terminal alkene coupling partner (see Figure 1.5).

The most effective molarity range of Grubbs second generation catalyst **1.17** is in the region of 5 mol% to 20 mol% relative to the alkene substrate.¹⁷ Lower equivalents of catalyst **1.17** generally result in sluggish reactions with very low yields of product, however higher equivalents can give rise to side products in which the benzyldiene group from the catalyst is transferred to the alkene substrate.

Isolation and purification of the olefin product from reactions involving catalysts **1.10** and **1.17** is often difficult due to the presence of residual ruthenium. As such a number of experimental techniques have been developed to aid catalyst by-product removal. Georg and co-workers developed an effective method for removing catalyst **1.10** from crude RCM reaction mixtures. Here, the crude product is stirred in trimethylphosphine or dimethyl sulphoxide (DMSO) and the resulting complex is removed by filtration through silica gel.⁶⁴ Maynard and Grubbs reported a method in which residual ruthenium byproducts from **1.10** are removed as water soluble ruthenium tris(hydroxymethyl) phosphine complexes.⁶⁵ However, this method has the draw back that many equivalents of the expensive phosphine ligand are required. Residual ruthenium, and other highly coloured impurities, can also be effectively removed from crude reaction mixtures by oxidation with a small amount of $\text{Pb}(\text{OAc})_4$ followed by filtration through a silica plug.⁶⁶ Such experimental techniques for the removal of residual ruthenium from crude reaction mixtures are somewhat redundant with the advent of catalysts such as **1.20**, as this catalyst can be separated from reaction mixtures by silica gel based chromatography for recycling. The growing number of solid-phase metathesis catalysts such as **1.26**, **1.27**, and **1.28** also reduces the need for extra purification measures, as alkene products can be washed from the solid-supported catalyst.

Various additives have been used to enhance metathesis reactions, for example CuCl , $\text{Ti}(\text{OiPr})_4$, and $\text{F}_3\text{CCO}_2\text{Li}$ have been used in RCM reactions of modified peptides.⁶⁷ The role of CuCl in enhancing RCM is not completely understood, but it is believed that complex formation with the ruthenium species results in a more stable active catalyst. The use of $\text{Ti}(\text{OiPr})_4$ or $\text{F}_3\text{CCO}_2\text{Li}$ as an additive has been shown to increase RCM product yields of peptide-based substrates.⁶⁷ This is proposed to be due to the co-ordination of titanium or lithium to the carbonyl functions of the peptide, thereby preventing undesirable carbonyl interaction with the catalyst.

Microwave-promoted metathesis reactions have also been utilized as an alternative to room temperature or thermally heated reaction conditions.³ Microwave energy allows for shorter reaction times and lower catalyst loadings. RCM of tri- and tetra-substituted alkene substrates containing electron-withdrawing groups has been shown to be far superior using microwave reaction conditions.⁶⁸ The effect of thermal versus non-thermal microwave effects on enhanced reaction times and yields has been studied.⁶⁹ Recently, solvent-free

microwave-assisted RCM reactions have also been reported,⁷⁰ further adding to the “green chemistry appeal” of olefin metathesis. In this thesis, a cyclic lysine-based compound (**4.6**) was synthesised using microwave conditions.

A number of these optimized conditions were used in metathesis reactions carried out in this thesis. These included the use of 20 mol% of catalyst **1.17** in refluxing DCM, along with a gentle flow of argon to remove the ethylene byproduct from the reaction mixture. Following metathesis the crude reaction mixtures were treated with 50 equivalents of DMSO relative to catalyst **1.17** to aid in the removal of catalyst by-products.

1.5 CROSS METATHESIS

Early reports of CM involved coupling of allyl methyl sulfide with unfunctionalised alkenes using a tungsten-carbene complex,²⁸ or the CM of styrene using Schrock's catalyst **1.1**.⁷¹ Since this time CM has evolved into a highly practicable synthetic tool due to the availability of catalysts that provide excellent functional group tolerance, good product yields, and an ability to operate under mild reaction conditions (see section 1.3 for catalyst discussion).

A limiting factor in the application of CM to many synthetic processes is the lack of predictability of product selectivity and stereochemistry. A general model for selectivity in CM was reported by Grubbs and Chatterjee *et al* in 2003.⁷² This model, summarised in Table 1.1, groups alkenes into four Types based on their relative ability to homodimerise and the susceptibility of their homodimers towards secondary CM reactions. This model also related the olefin Type to reactivity with either catalyst **1.17** or **1.10**, which can allow for selectivity in a CM reaction based on advantageous catalyst choice.

Olefins of Type I (e.g. sterically unhindered, electron-rich) undergo rapid metathesis to form homodimers, which can then participate in secondary CM with another olefin. Type II olefins have slower homodimerisation rates than Type I olefins, and homodimers of this type rarely participate in secondary metathesis reactions. Type III olefins are unable to form homodimers in the presence of **1.17**, **1.10**, or **1.1** but can undergo CM with olefins of Type I or Type II. Type IV olefins (e.g. sterically hindered, electron-deficient) can not participate in CM reactions with these catalysts, but do not have a detrimental effect on catalyst activity.

Olefin Type	Grubbs 2 nd generation 1.17	Grubbs 1 st generation 1.10	Schrocks 1.1
Type I	Terminal olefins, 1° allylic alcohols, allyl boronate esters, styrenes (no large ortho. substit.) esters, allyl halides, allyl silanes, allyl phosphonates, allyl silanes, allyl phosphine oxides, allyl sulfides, protected allyl amines	Terminal olefins, allyl silanes, 1° allylic alcohols, ethers, esters, allyl boronate esters, allyl halides	Terminal olefins, allyl silanes
Type II	Styrenes (large ortho. substit.), acrylates, acrylamides, acrylic acids, acrolein, vinyl ketones, unprotected 3° allylic alcohols, vinyl epoxides, 2° allylic alcohols, perfluorinated alkane olefins	Styrene, 2° allylic alcohols, vinyl dioxolanes, vinyl boronates	Styrene, allyl stannanes
Type III	1,1-disubstituted olefins, non-bulky trisub. olefins, vinyl phosphonates, phenyl vinyl sulfone, 4° allylic carbons, 3° allylic alcohols (protected)	Vinyl siloxanes	3° allyl amines, acrylonitrile
Type IV	Vinyl nitro olefins, trisubstituted allyl alcohols (protected)	1,1-disubstituted olefins, disub. α,β -unsaturated carbonyls, 4° allylic carbo-containing olefins, perfluorinated alkane olefins, 3° allyl amines	1,1-disubstituted olefins

* Table adapted from Grubbs and Chatterjee *et al.*⁷² See references therein for examples of CM reactions with above functional groups.

Table 1.1. Model for olefin selectivity in CM

Reaction between equal amounts Type I olefins results in a statistical distribution of metathesis products. The yield of the cross-coupled $R^1CH=CHR^2$ product can be improved by using more equivalents of one of the Type I olefin substrates, for example $R^1CH=CH_2$, as shown in Figure 1.5.

	$=R^1:=R^2$	CM product yield
	1:1	50%
	2:1	66%
	4:1	80%
	10:1	91%
	20:1	95%

Figure 1.5. Statistical distribution of CM products of olefin Type I

Olefins of different Types can react in an equi-molar fashion to selectively produce a higher cross-coupled product yield, compared to equi-molar reaction of olefins of the same Type. For example in a reaction between a Type I and Type II olefin, although the Type I olefin may initially homodimerise, the type I homodimer then undergoes secondary metathesis with the Type II olefin to form the cross-coupled product.

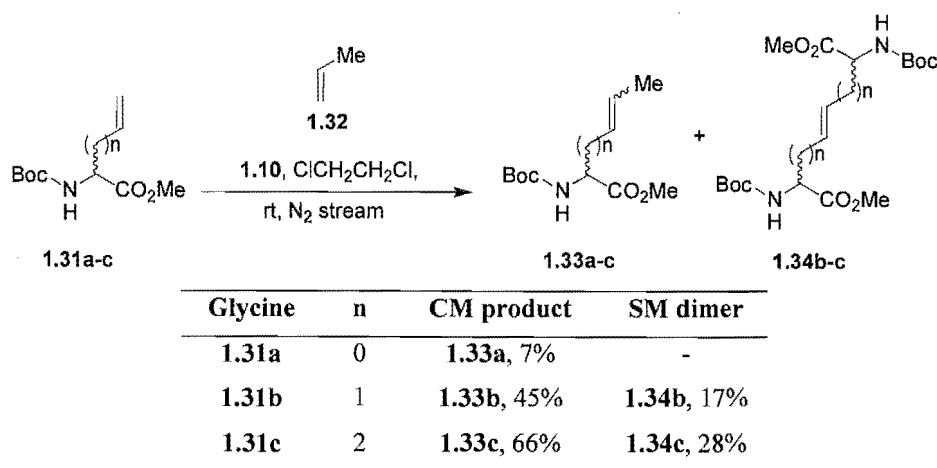
1.5.1 Application of cross metathesis to modified amino acids

Work in this thesis describes the use of CM to amino acids suitably substituted with an olefin functionality. What follows is a summary of previous work involving the use of CM coupling reactions with various derivatised amino acids.

CM has attracted considerable attention as a means to prepare modified amino acids and peptidomimetics that possess useful chemical and biological properties. CM reactions with amino acid substrates can be categorized based on the position of the olefin substitution. For example, a substituent bearing a terminal double bond can be attached at either the (i) α -carbon, (ii) amino acid side chain, (iii) amino-terminus, or (iv) carboxyl terminus of an amino acid. Reactions can be further classified on the basis of the type of amino acid used.

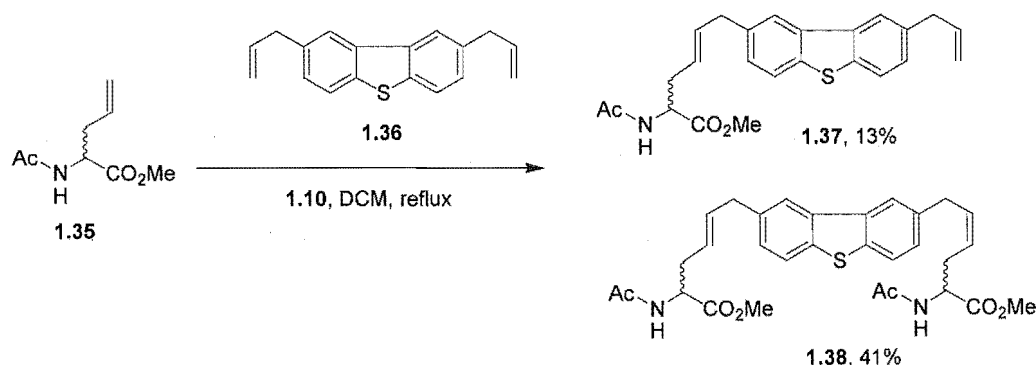
α -Carbon Substituted Amino Acids

Modified glycine has proven to be a popular scaffold for CM chemistry. The first examples of CM using amino acid-based substrates were published by Gibson and co-workers in 1997, where cross-coupling to styrene was described.³³ This was followed soon after by further systematic studies involving CM of glycine-based **1.31a-c** to propene **1.32** (Scheme 1.1).³² A four-chain alkene tether as in **1.31c** gives the highest cross-coupled yield.



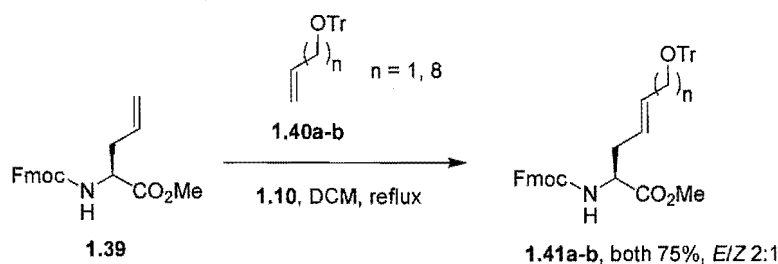
Scheme 1.1.

Heterocycles have also been successfully coupled to allyl glycine. For example, racemic methyl *N*-acetylallylglycinate **1.35** was coupled to 2,8-diallyldibenzothiophene **1.36** as a key step in the preparation of antibiotic agents **1.37** and **1.38** (Scheme 1.2).⁷³



Scheme 1.2.

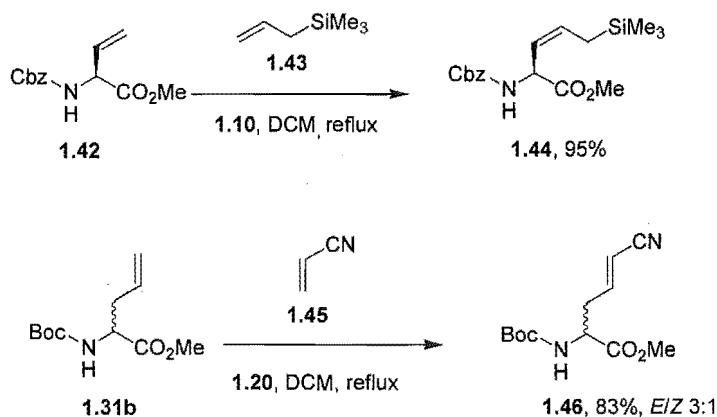
CM reactions have been used as a preliminary step in developing methodology for the catalytic cyclisation/cleavage of tetrapeptide-derived macrocycles from solid supports.⁷⁴ Here, the side-chain of Fmoc-allylglycine methyl ester **1.39** was selectively coupled to *O*-trityl-protected alkenols **1.40a-b** of differing chain lengths (Scheme 1.3). This gave rise to CM products **1.41a-b** that were subsequently deprotected, resin-linked, and incorporated into tetrapeptides.



Scheme 1.3.

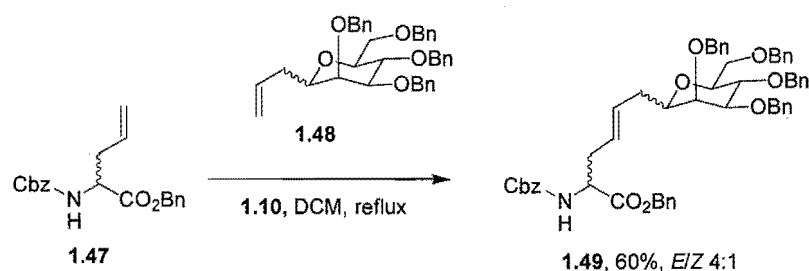
CM of allylsilane substrates gives rise to CM products that can be further elaborated via the silyl group. In one example, protected vinyl glycine **1.42** was functionalised using allyl trimethyl silane **1.43** in excellent yield and with good stereoselectivity (Scheme 1.4).⁷⁵ More recently, Blechert and co-workers employed the second generation catalyst **1.20** for a

highly selective CM of protected amino acid **1.31b** and acrylonitrile **1.45** - reactions that could previously only be achieved using sensitive molybdenum catalysts (Scheme 1.4).⁴²



Scheme 1.4.

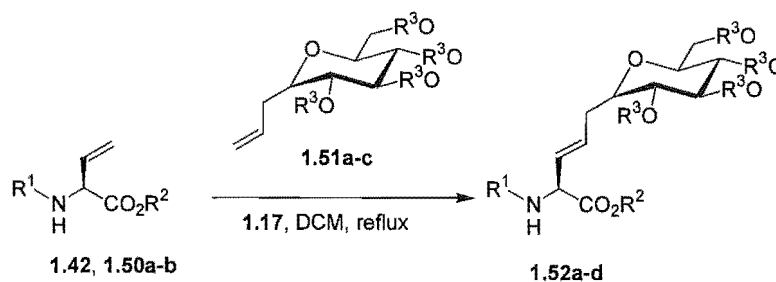
Roy and co-workers carried out CM of protected allylglycine **1.47** with suitably substituted monosaccharide **1.48** to give *C*-linked carbohydrate **1.49** that possesses stability towards enzymatic and metabolic cleavage (Scheme 1.5).⁷⁶ Reactions of this type provide convenient access to important glycopeptidomimetics, where the parent glycopeptides are known to play an important role in a number of important biological processes including tumor metastasis and chemotaxis. A series of glycoamino acids (**2.80** – **2.85**) were prepared in this thesis using CM coupling, as is described in Chapter 2.



Scheme 1.5.

Nolen and colleagues have reported the synthesis of *C*-glycosyl amino acids in good yields using Grubbs second generation catalyst **1.17** (Scheme 1.6).⁷⁷ Here, CM of tetra-*O*-protected-glucose substrates **1.51a-d** with vinylglycine derivatives **1.42** and **1.50a-b**

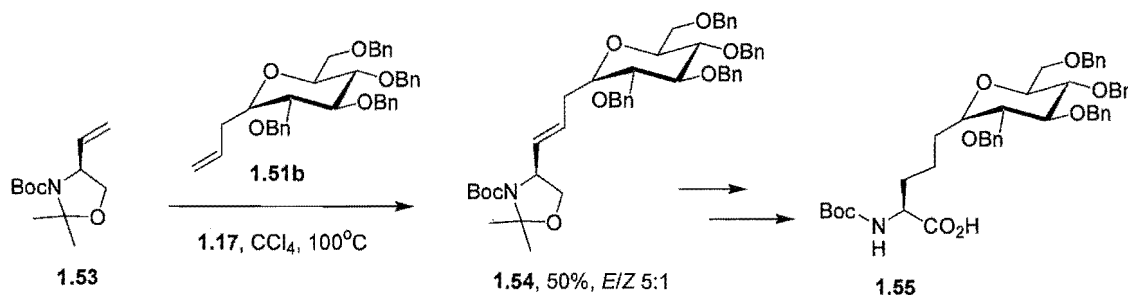
proceeded efficiently, and the products obtained (**1.52a-d**) were hydrogenated to give C-glycosyl asparagines as models for use in enzyme assays.



Vinyl glycine	R ¹	R ²	Carbohydrate	R ³	CM product
1.42	Cbz	Me	1.51a	Ac	1.52a, 82%
1.42	Cbz	Me	1.51b	Bn	1.52b, 64%
1.50a	Boc	Bn	1.51c	Ac	1.52c, 94%
1.50b	Fmoc	Bn	1.51c	Ac	1.52d, 77%

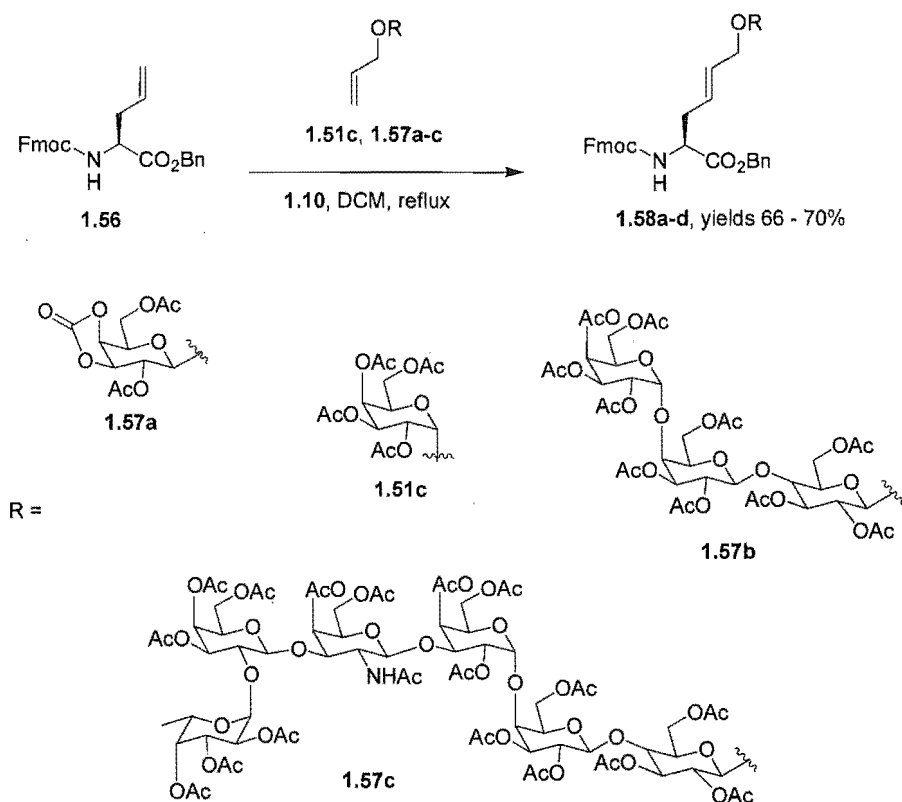
Scheme 1.6.

C-Glycosyl amino acids can also be prepared by reaction of *N*-Boc-vinylloxazolidine **1.53**, a vinylglycine equivalent, and sugar **1.51b** using Grubbs second generation catalyst **1.17**, where **1.10** does not catalyse these reactions (Scheme 1.7).⁷⁸ The resulting metathesis product (**1.54**) was hydrogenated, acylated, and the oxazolidine ring oxidatively cleaved to afford a versatile building block (**1.55**) for the synthesis of modified glycopeptides. CM of vinyl glycine derivatives with allylated sugars has also been carried out by Ben and Liu, in a study directed towards the development of recrystallisation inhibitors.⁷⁹



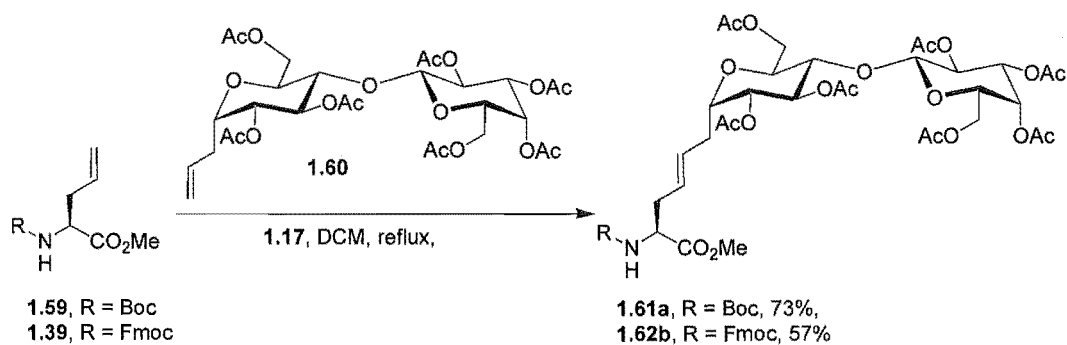
Scheme 1.7.

Danishefsky and co-workers have synthesised glycosyl amino acids **1.58a-d** via CM, which are suitable for incorporation into polymeric antitumour vaccines (Scheme 1.8).⁸⁰ Here, Fmoc-*L*-allylglycine benzyl ester **1.56** was coupled to *O*-allyl glycosides **1.51c** and **1.57a-c** to give the desired CM products in good yields.



Scheme 1.8.

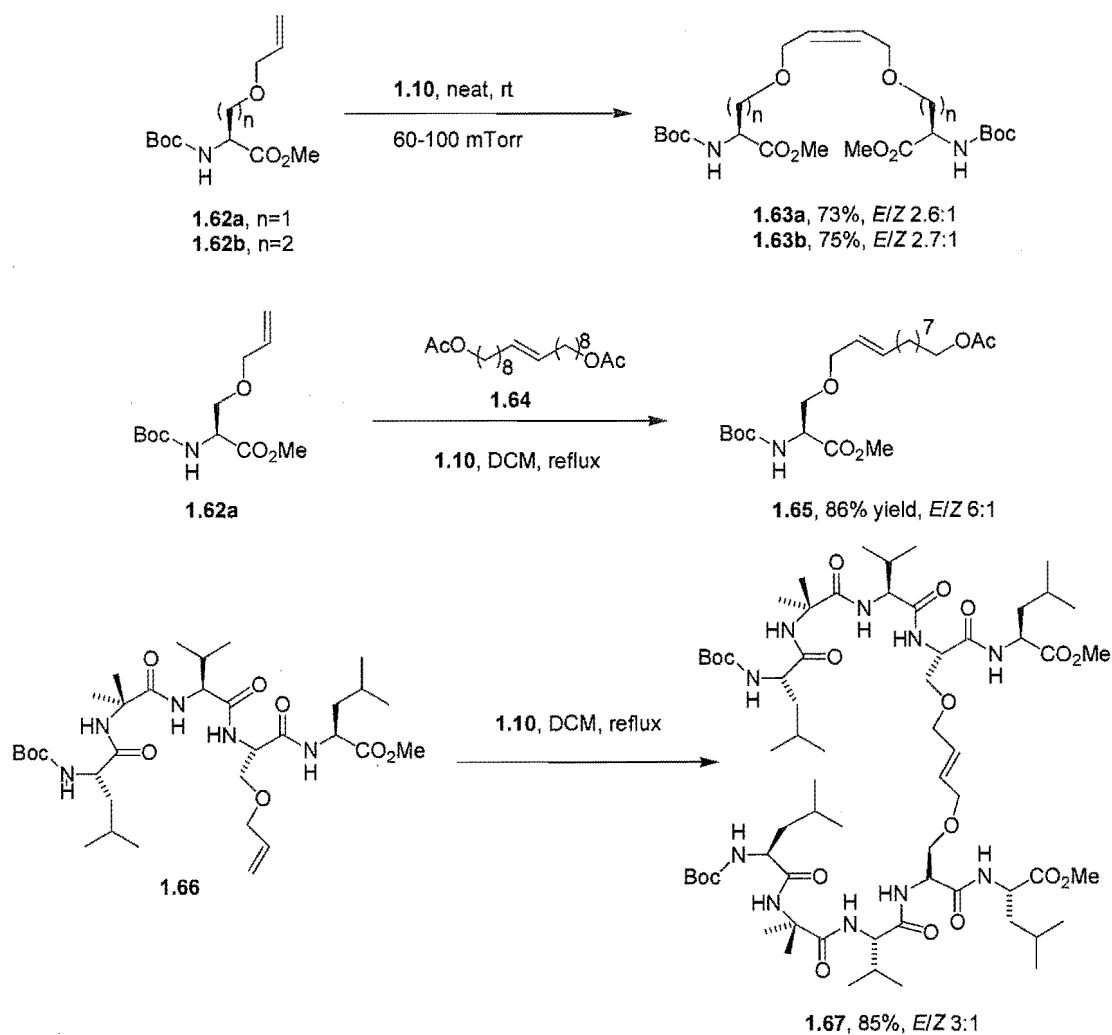
Other reports on the synthesis of stable glycopeptide analogues using CM methodology have appeared with a view to identify potential therapeutic agents.⁸¹ For example, protected *C*-allyl glycoside **1.60** has been coupled to protected allylglycines **1.59** and **1.39** using Grubbs second generation catalyst **1.17** - introduced in two equal portions at 24 hour intervals - to give moderate-to-good overall yields of coupled products **1.61a-b** (Scheme 1.9). This initial work was extended to allow the conjugation of *C*-allyllactose as a first step in the development of a post-translational glycopeptide synthetic strategy.



Scheme 1.9.

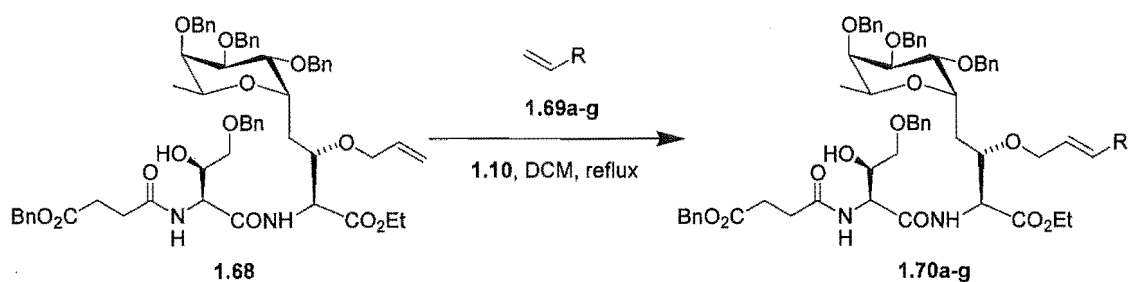
Side Chain Substituted Amino Acids

The amino acid serine has been used as a basis for side-chain installation of an olefin tether, to give a substrate suitable for CM. Grubbs and colleagues have explored a number of examples of CM reactions of serine derivatives with both terminal and substituted alkene coupling partners.⁶³ Allyl ethers of protected serine **1.62a** and homoserine **1.62b** were dimerized by CM under reduced pressure to give dimeric **1.63a-b**, while treatment of Boc-*L*-serine(*O*-allyl) methyl ester **1.62a** with bis(9-nonenyl acetate) **1.64**, itself made by CM, generated lipophilic amino acid **1.65** in high yield and with good stereoselectivity (Scheme 1.10). Larger and more complex architectures were also shown to be compatible with CM chemistry, for example hydrophobic pentapeptide framework **1.66** (Scheme 1.10).⁶³ The dimerized pentapeptide **1.67** represents an example of side chain to side chain cross linking via a non-native carbon-carbon linkage. The application of CM to the formation of amino acid dimers was also investigated in this thesis, and this work is discussed in Chapter 3.



Scheme 1.10.

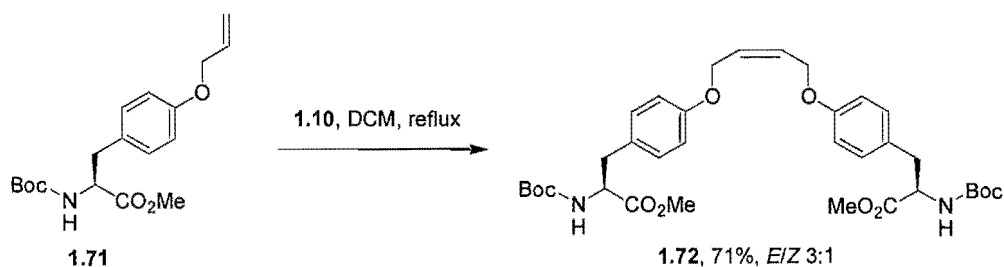
Aryl substituted *C*-fucopeptides **1.70a-g** have been synthesised using CM methodology (Scheme 1.11).⁸² These products are important in that they mimic tetrasaccharide sialyl Lewis X, a carbohydrate-based terminal unit found in cell-surface glycoproteins and glycolipids, which interacts with E- and P-selectin to mediate the early stages of an inflammatory response. It was found that CM at room temperature failed to give the desired coupled products. However, reaction between **1.68** and **1.69a-g** at reflux afforded an array of CM products in reasonable yields, with the electron poor pentafluorostyrene giving the lowest yield. The authors noted that activated aromatic and non-aromatic olefins gave a mixture of *E* and *Z* olefins, with non-activated aromatic olefins strongly favouring the *E* olefin.



R	CM yield (%)	E/Z ratio
Ph	74	>95:5
Bn	65	72:25
4-MeOC ₆ H ₄	79	87:13
C ₆ F ₅	17	70:30
2-Naphthyl	71	>95:05
2-Fluorenyl	70	>95:05
4-Binaphthyl	73	>95:05

Scheme 1.11.

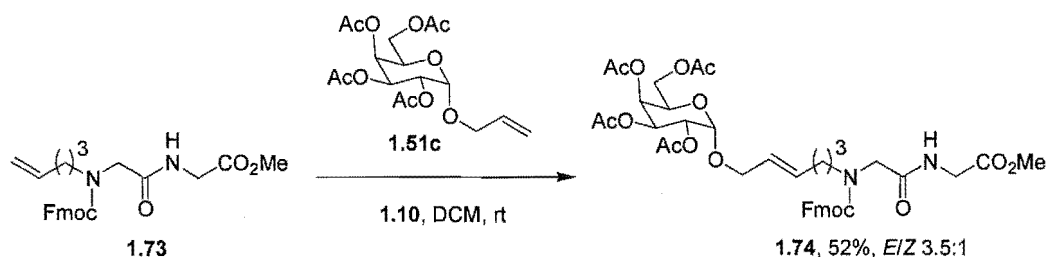
Tyrosine-based systems have also been used in CM dimerisation reactions, where the *O*-allyl tether of **1.71** is of sufficient length to allow CM reactions to proceed to afford dimeric **1.72** (Scheme 1.12).⁶³ By contrast, the analogous dimerisation of Boc-*L*-allylglycine methyl ester **1.59** does not proceed well, where the close proximity of the double bond to the amino acid backbone appears to hinder catalyst binding.



Scheme 1.12.

N-Terminus Substituted Amino Acids

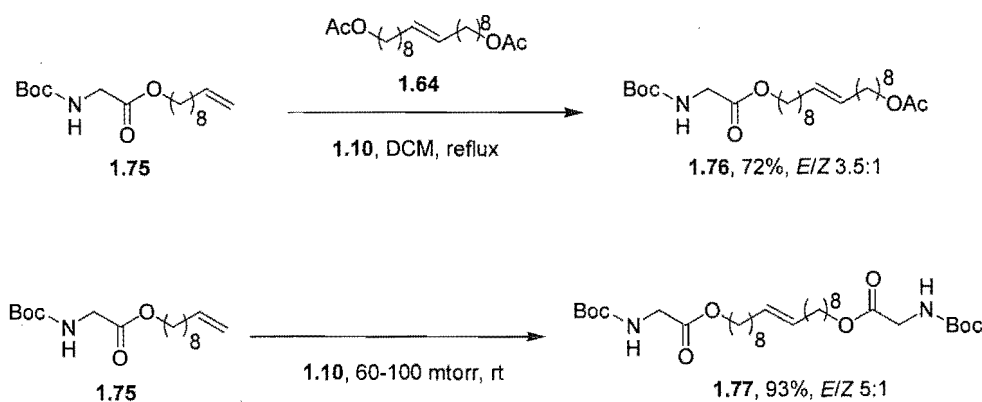
There are comparatively far fewer examples of CM reactions of an olefin attached to the α -nitrogen of an amino acid or peptide. Nevertheless, the CM of *N*-alkenylpeptoids **1.73** and *O*-allyl glycoside **1.51c** has been reported by Hu and Roy to give glycopeptide **1.74** (Scheme 1.13).⁸³ The reaction is tolerant of free carboxylic acids, but not of secondary amine functionalities. It was found that a shorter tether between the nitrogen and the double bond results in a lower yield of the desired CM product.



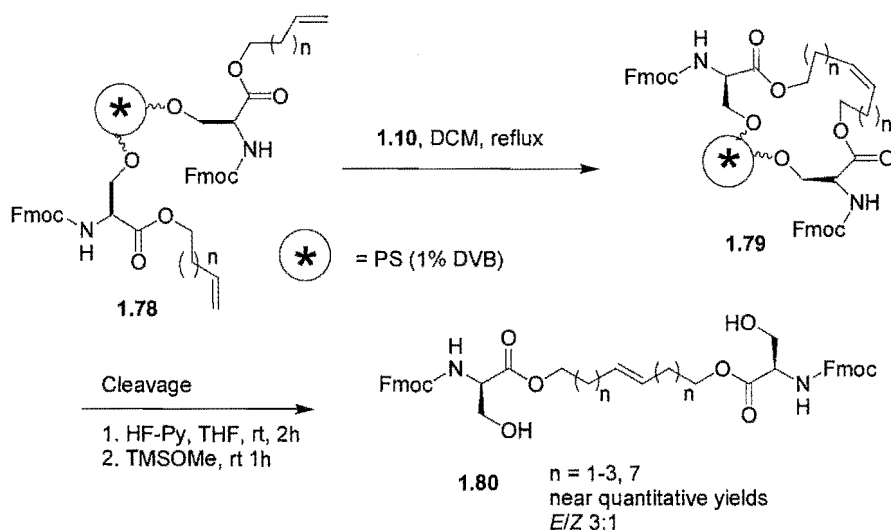
Scheme 1.13.

C-Terminus Substituted Amino Acids

CM at the *C*-terminus of suitably substituted amino acids and peptides has also been reported. For example, 9-decen-1-yl Boc-glycinate **1.75** reacts with 9-decen-1-yl acetate homodimer **1.64** in the presence of catalyst **1.10**, to give a differentially functionalized 9-octadecene-1,18-diol **1.76** (Scheme 1.14).⁶³ Glycine derivative **1.75** also undergoes self-metathesis to give amino acid homodimer **1.77** in excellent yield.

**Scheme 1.14.**

C-Terminally substituted CM reactions have also been extended to the solid phase using a polystyrene (1% DVB) resin.⁸⁴ These resin-bound amino acids and peptides can be chemically manipulated and subsequently cleaved from the resin. Schreiber and colleagues also report solid phase “intra-site” CM reactions, in near quantitative yields, using individual polystyrene polymer beads with a silyl linker (Scheme **1.15**).⁸⁵ It was found that the alkyl chain length of **1.78** had little effect on the reaction efficiency. Here, the metathesis step to give **1.79** is effectively a RCM reaction, and once the product is cleaved from the resin gives the analogous solution phase CM product (**1.80**).

**Scheme 1.15.**

1.6 RING-CLOSING METATHESIS

Currently RCM is the most commonly used type of olefin metathesis transformation. A number of reviews have been written outlining the reaction scope and wide range of substrates that have been constructed using RCM – these include a review on the application to heterocycles, alkaloids, and peptidomimetics,⁸⁶ rigidified amino acids and peptides,⁸⁷ advances in organic synthesis,⁸⁸ relay RCM for sterically hindered substrates,⁸⁹ and cyclic peptides.⁹⁰ Reviews have also been written that discuss the synthesis of oxygen- and nitrogen-containing,⁹¹ or phosphorus and sulfur containing⁹² heterocycles via RCM.

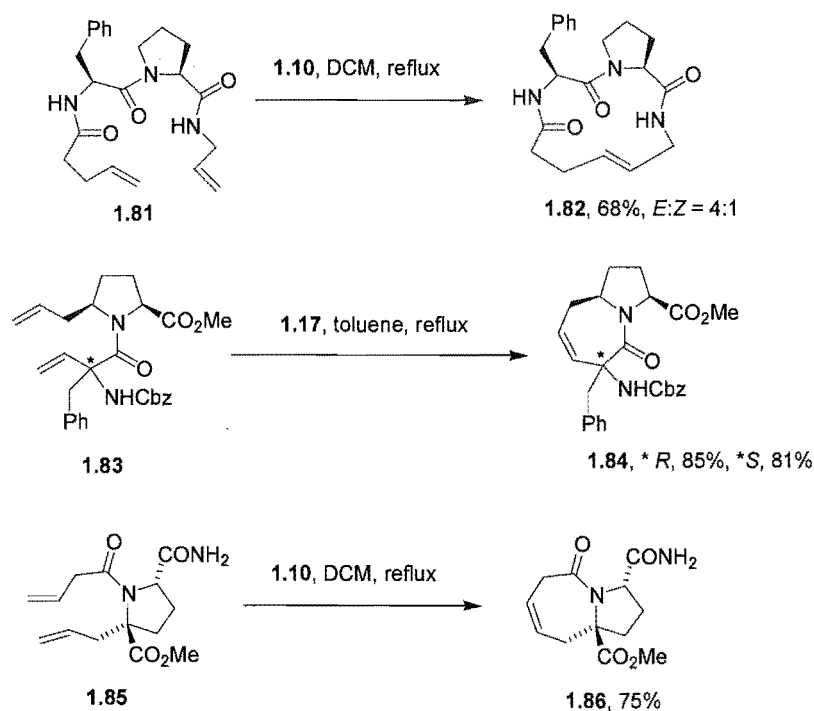
1.6.1 Application of RCM to modified amino acids and peptides

The use of peptides in drug discovery and therapeutics is often limited by their poor bioavailability and proteolytic degradation. RCM is used in the construction of peptidomimetics* for two reasons^{93,94} – 1) the incorporation of a double bond to help prevent proteolytic cleavage and assist *in vivo* stability; 2) to restrain the structure into a biologically advantageous conformation, for example a β -turn conformation. Cyclic peptidomimetics can be synthesised in many ways other than using RCM, for example by peptide bond formation.⁹⁵⁻⁹⁷ In this thesis the application of RCM to side-chain modified amino acids and peptides is examined. What follows are selected literature examples demonstrating the use of RCM on various modified amino acids and peptides, illustrating the potential scope of this reaction.

The development of small molecule peptidomimetics based around the β -turn Pro-Phe dipeptide are useful as therapeutics, as this is the peptide bond that is cleaved by HIV-I protease. Inhibition of this protease has been shown to be a promising treatment against the HIV virus.⁹⁸ Iqbal and co-workers synthesised the β -turn mimic **1.82** from pent-4-enoyl-*L*-Phe-*L*-Pro-*N*-allyl amide **1.81** (Scheme 1.16).⁹⁹ The importance of chirality of the phenylalanine residue was demonstrated by the fact that RCM did not take place using 4-enoyl-*D*-Phe-*L*-Pro-*N*-allyl dipeptide. This suggests that acyclic diene **1.81** contains some

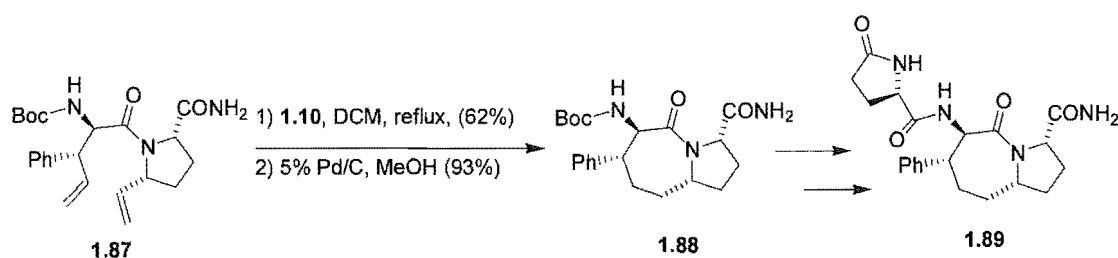
* A peptidomimetic is defined as a non-peptide substance that has an analogous structural and/or secondary structure to a parent peptide, that allows it to behave in biological systems in a likewise fashion.

structural pre-organisation that favours RCM. Fused bicyclic lactam **1.84** has been enantiomerically prepared by RCM of **1.83**, which was also designed as a β -turn Pro-Phe dipeptide mimic.¹⁰⁰ Conformational analysis of **1.84** by NMR and MD calculations confirmed a well-defined β -turn mimic conformation. Proline-based lactam-bridged type VI β -turn model systems such as **1.86** have also been synthesised by Gmeiner and colleges.¹⁰¹ Here three cyclic structure **1.86** was prepared in good yield by RCM of **1.85**.



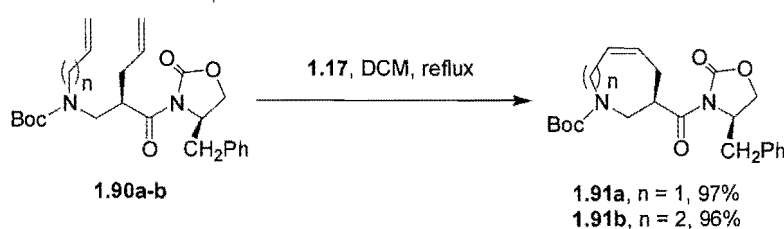
Scheme 1.16.

Moeller *et al* have devised a general strategy for the synthesis of bicyclic lactam peptide building blocks by RCM in an effort to build constrained peptidomimetics of the endocrine hormone thyroliberin.¹⁰² Diene **1.87** was submitted to RCM to give the cyclic product in good yield, and this was hydrogenated to afford lactam **1.88** (Scheme 1.17). Lactam **1.88** was coupled to pyroglutamic acid in several steps to give **1.89**. This was used in biological studies to investigate the preferred binding conformation at the endocrine receptor TRH-R.



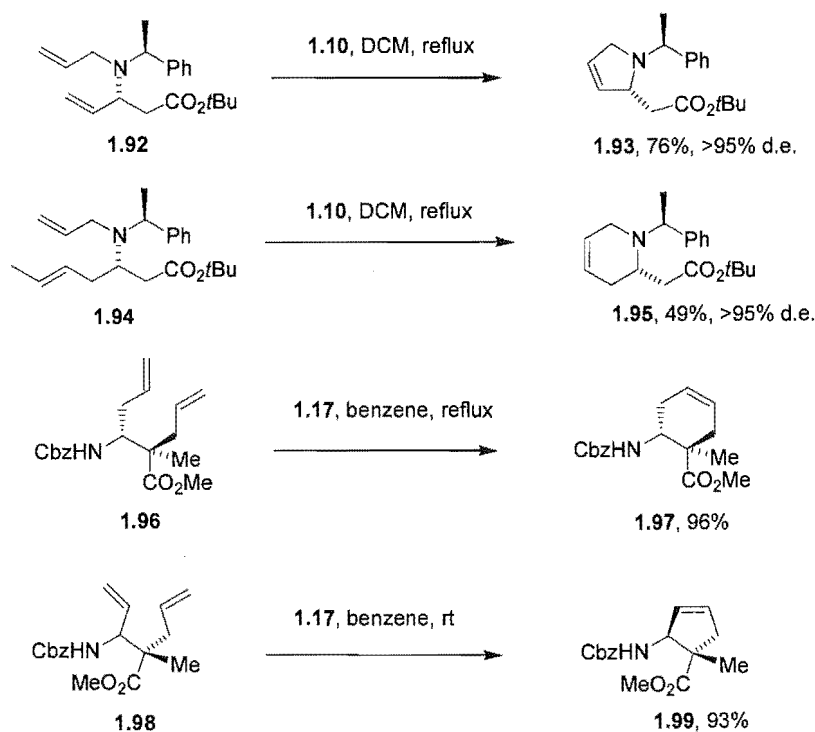
Scheme 1.17.

Yamanaka and colleagues synthesised cyclic mimics of a β -turn sub-structure of Arg-Gly-Asp on route to non-peptide platelet aggregation inhibitors (Scheme 1.18).¹⁰³ 7- And 8-membered containing **1.91a-b** were prepared in good yields from β -amino acid-type precursors **1.90a-b**, both in enantiomerically pure form. The oxazolidinone chiral auxiliary is subsequently cleaved from **1.91a-b** to give the target compounds.



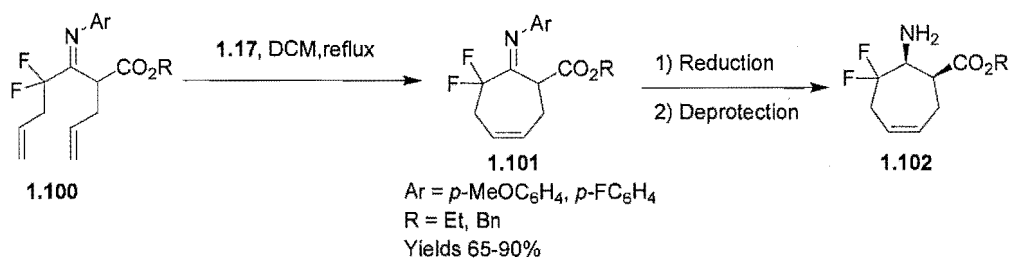
Scheme 1.18.

A range of cyclic β -amino acids have been synthesised using RCM. Pyrrolidine **1.93** and piperidine **1.95** β -amino esters have been synthesised by RCM of acyclic precursors **1.92** and **1.94** (Scheme 1.19).¹⁰⁴ Compounds such as **1.93** and **1.95** are useful building blocks in the synthesis of complex natural products containing β -amino acid functionalities. Abell and colleagues have reported a versatile method for the preparation of functionalised cyclic β -amino acid esters from methionine, allylglycine, and serine starting materials.¹⁰⁵ β -Amino acid ester diene **1.96**, synthesised itself from allyl glycine, underwent RCM to afford 6-membered derivative **1.97** in high yield (Scheme 1.19). Diene **1.98**, synthesised itself from methionine in several steps, afforded the RCM product **1.99** that contained a 5-membered ring also in very high yield.



Scheme 1.19.

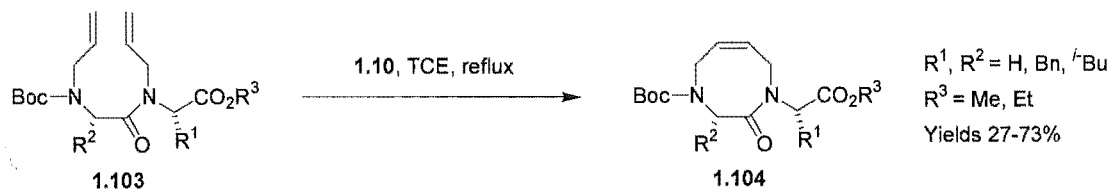
Fustero and co-workers have applied RCM chemistry to the diastereoselective synthesis of seven-membered difluorinated β -amino acid derivatives.¹⁰⁶ Fluorinated β -imino esters **1.100** were cyclised to give **1.101**, the imine reduced to give exclusively the *cis*-amino isomer, and then amino deprotected to give fluorinated β -amino acid mimics **1.102** (Scheme 1.20).



Scheme 1.20.

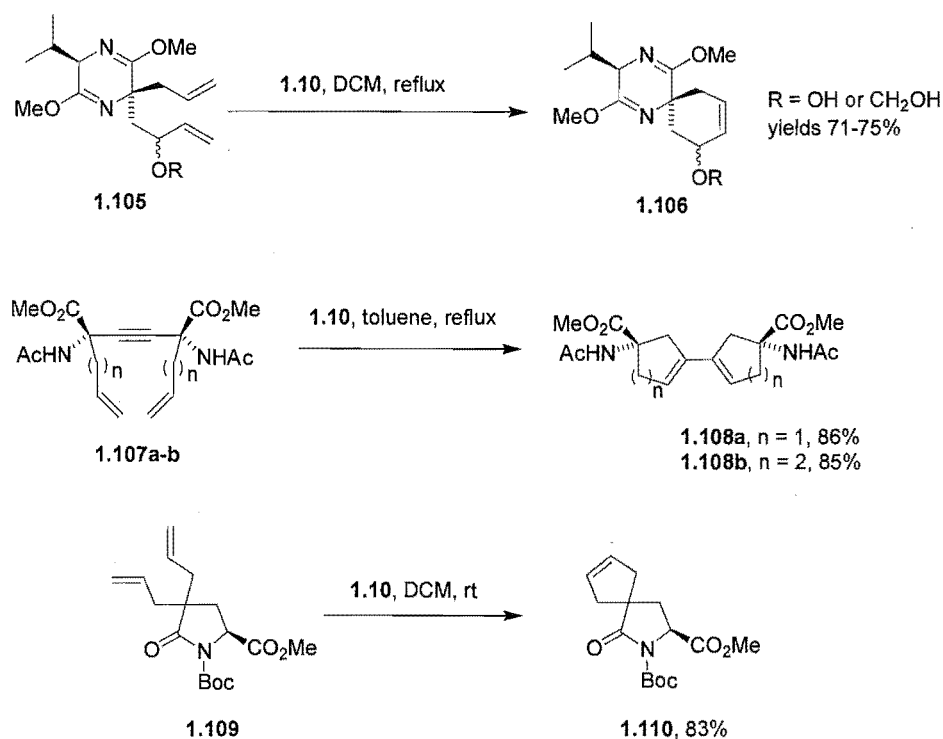
Reichwein and Liskamp synthesised various cyclic dipeptides using an amide-to-amide RCM cyclisation strategy: *N*-diallylated diene **1.103** was cyclised using RCM to afford

constrained peptides **1.104** that contained an 8-membered ring. The yields of cyclic products were dependent on the different substituents used (Scheme 1.21).⁶⁷



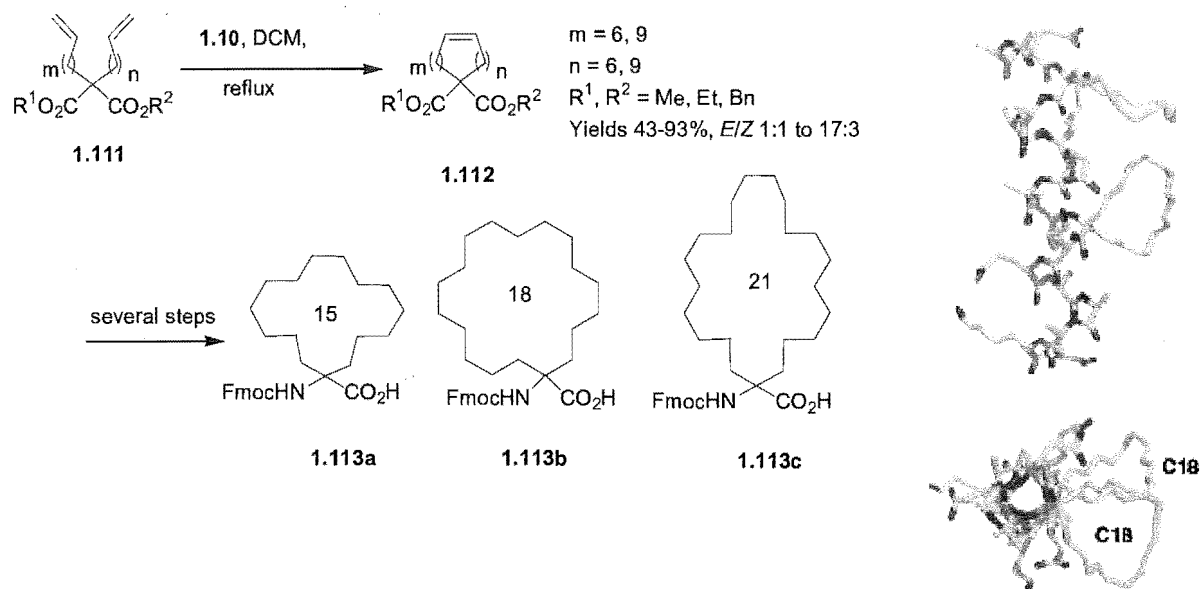
Scheme 1.21.

Spiroannulated cyclic dipeptides **1.106** have been synthesised by Efskind *et al* as precursors of conformationally restricted cyclic α -amino acids (Scheme 1.22).¹⁰⁷ The same authors also report a RCM cascade reaction in the formation of two-ring bisamino acid derivatives **1.108a-b** that are interconnected by a C_4 - C_6 bridge.¹⁰⁷ Spirocyclopentane **1.110**, designed as a rigid dipeptide mimic, has been synthesised by Scolastico and co-workers using RCM from diene **1.109** in good yield (Scheme 1.22).¹⁰⁰



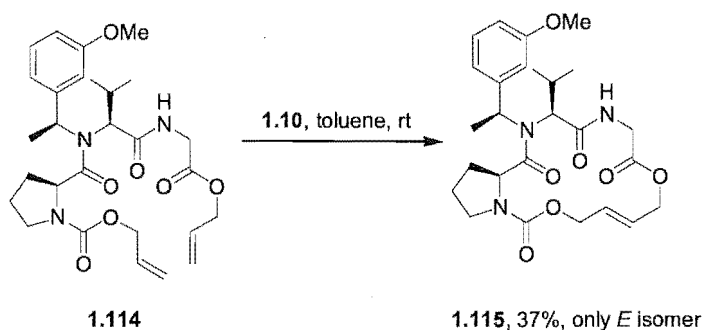
Scheme 1.22.

α,α -Disubstituted glycines that bear a large hydrophobic ring have been synthesised using RCM.¹⁰⁸ The RCM reactions were carried out on malonate derivatives **1.111** to give cyclic products **1.112**, with the amide functionality subsequently introduced in several steps to give **1.113a-c** (Scheme 1.23). The stability of an α -helical 17-amino acid peptide was enhanced by the replacement of two alanine residues with two Fmoc α,α -disubstituted cyclic residues **1.113b** bearing a cyclic 18-membered ring.



Scheme 1.23.

The tripeptide allylic ester **1.114** undergoes RCM to give novel 16-membered cyclic-based structure **1.115** (Scheme 1.24).¹⁰⁹ The tripeptide RCM substrate **1.114** was itself synthesised in single step in an Ugi reaction.



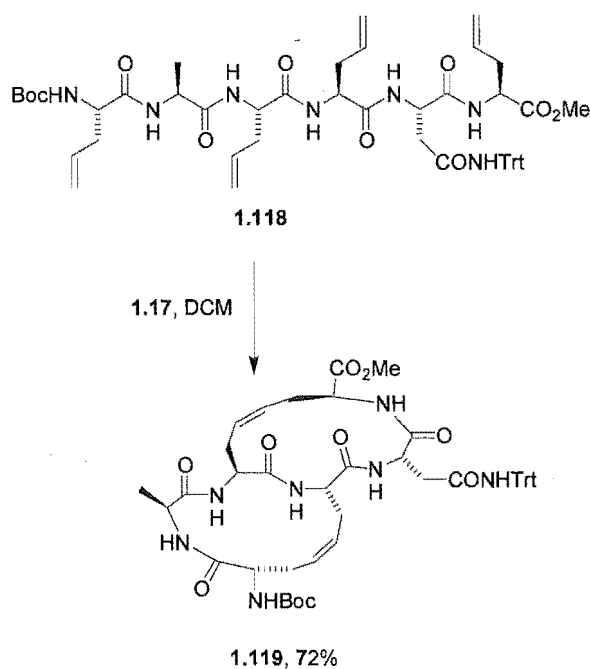
Scheme 1.24.

A bicyclic side chain knotted pentapeptide has been constructed by Liskamp and colleges, to mimic the structure of the glycopeptide antibiotic vancomycin (refer to **2.1**, Figure **2.1**). Bis-RCM precursor **1.116**, containing a central di-alkylated aromatic core and two allylated serine residues, was cyclised in one step to give knotted peptide **1.117**, which contains the same side-chain connectivity as vancomycin (Scheme **1.25**).¹¹⁰ The RCM was carried out in the presence of a small amount on DMF because of the poor solubility of **1.116**. Consequently, a stoichiometric amount of catalyst **1.17** was required for RCM to occur since DMF also deactivates the ruthenium catalyst.

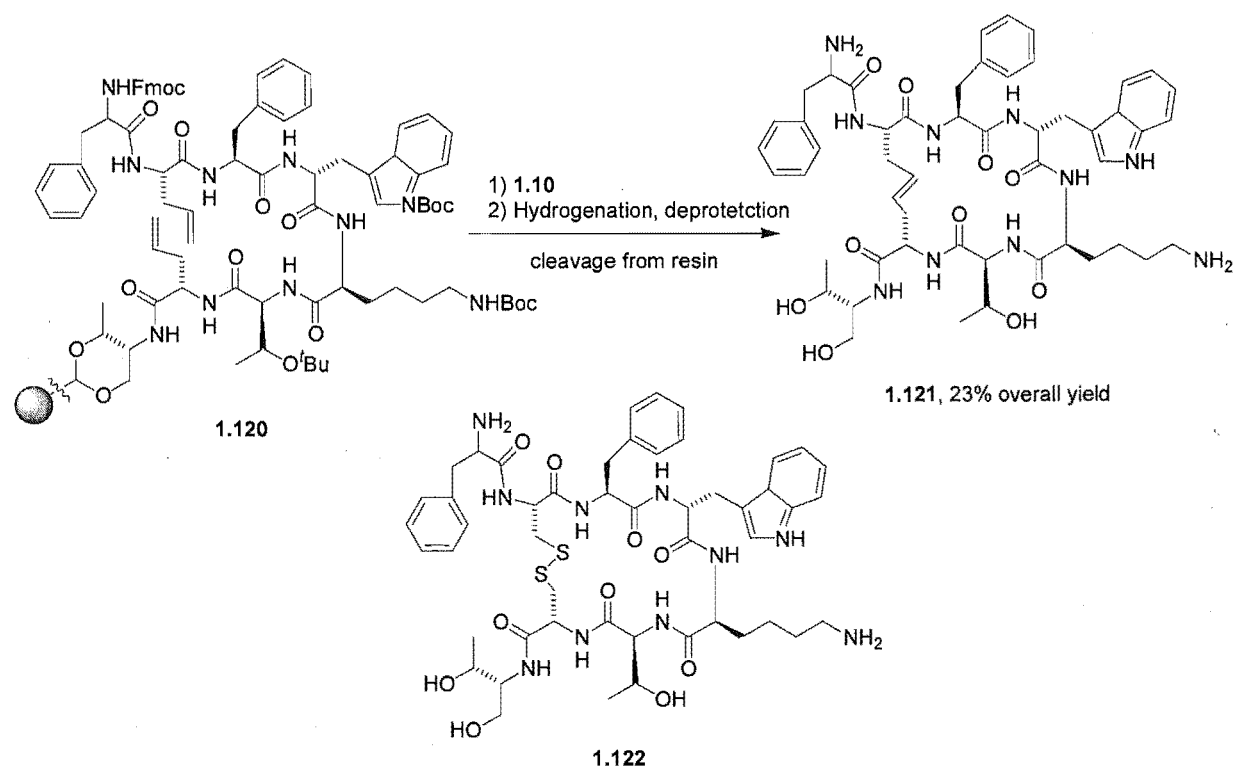


Scheme 1.25.

Liskamp and co-workers have synthesised a cross alkene-bridged hexapeptide in a single step with two RCM reactions.¹¹¹ The tethered amino acid side chains that cross over each other provide a mimic of the lantibiotic nisin Z, which contains a thioether “knot” of the same geometry. Protected peptide **1.118** was submitted to RCM with **1.17** and the desired bicyclic product **1.119** was obtained in 72% (Scheme **1.26**). The predominance of product **1.119** over other cyclic isomers was consistent with molecular mechanics calculations which demonstrated that the corresponding monocyclic precursors to **1.119** had lower energy intermediates than other possible RCM monocycles.

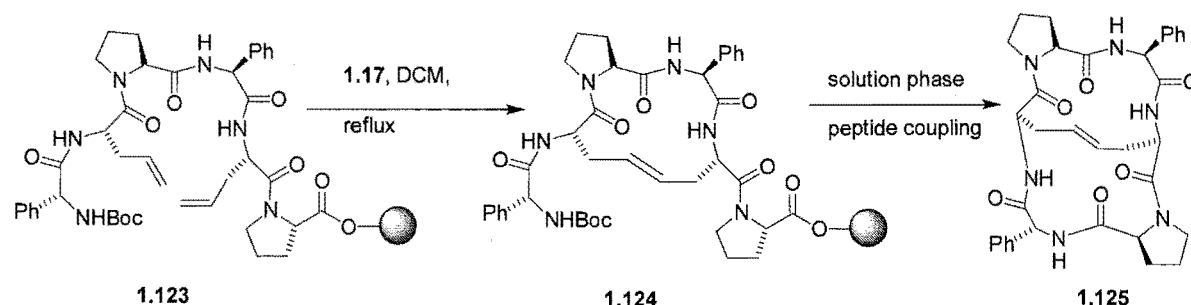
**Scheme 1.26.**

There are many instances in which a disulfide bond between thiol residues in cysteine-containing peptides has been mimicked using a C-C bond generated by RCM. The disulfide bridge of many peptides serves a skeletal role only, such that it can be replaced with a non-reducible structural mimic without significantly altering biological activity. For example, a synthetic analogue of the tetradecapeptide somatostatin **1.122** has been synthesised where the S-S bond was replaced by a C-C double bond (Scheme 1.27).¹¹² Resin-bound diene **1.120** underwent RCM, and was then hydrogenated, deprotected, and cleaved from the resin to give cyclic **1.121** in moderate overall yield.



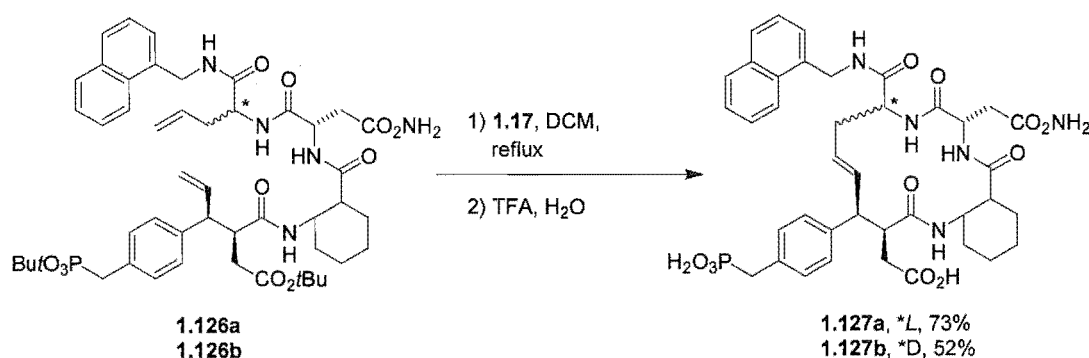
Scheme 1.27.

Bicyclic hexapeptides containing a carbon-carbon double bond bridge have been prepared. Diene **1.123**, assembled on solid-phase, underwent RCM to give cyclic **1.124** (Scheme 1.28).¹¹³ Monocyclic **1.124** was then cleaved from the resin and *N*- to *C*-terminal cyclisation was carried out in solution phase to give bicyclic hexapeptide **1.125**. Compounds of this type are rigid β -turn mimics, that contain two β -turn units and intrinsic C_2 -symmetry.



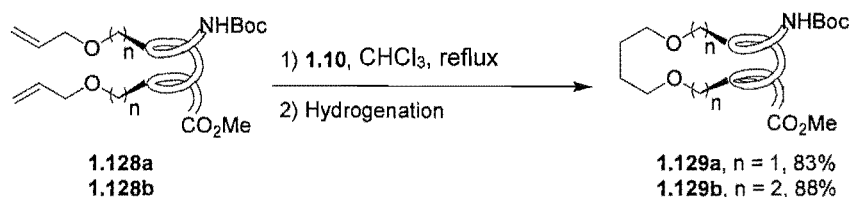
Scheme 1.28.

Burke and co-workers synthesised constrained (Grb) SH2 domain-binding peptides by RCM, where the introduced C-C bond mimics the bend conformation of the natural peptide. Dienes **1.126a-b** were cyclised using catalyst **1.17** in a high molar amount to give cyclic **1.127a-b** good yields (Scheme 1.29).¹¹⁴ Both **1.127a** and **1.127b** demonstrated significant potency in binding experiments to chip-bound Grb2 SH2 domain proteins compared to the analogous unconstrained peptides.



Scheme 1.29.

Amino acid side-chain to side-chain tethered peptides have been synthesised by Grubbs and colleges, based around the hydrophobic hexapeptide Boc-Val-Ala-Leu-Aib-Val-Ala-Leu-OMe that adopts a 3₁₀-helical conformation in CDCl₃.¹¹⁵ In the modified peptide, the Ala residues were both replaced with either *L*-serine *O*-allyl ether in **1.128a** or *L*-homoserine *O*-allyl ether in **1.128b** (Scheme 1.30). Dienes **1.128a-b** were then cyclised by RCM and the olefin hydrogenated to give stable helices containing 22-membered (**1.129a**) and 23-membered (**1.129b**) cyclic structures in excellent yields.



Scheme 1.30.

The many examples presented in this chapter illustrate the applicability of RCM to a wide variety of synthetic amino acid and peptide transformations. Further discussion on the benefits and advantages that RCM provides in the construction of peptidomimetics and therapeutics are discussed in Section 4.1, Chapter 4.

In summary, this chapter discusses the various types of metathesis reactions, in particular CM and RCM, and outlines the proposed mechanism of olefin metathesis. An overview of the development and properties of metathesis catalysts is also presented. Section 1.5 discusses the CM of amino acid-based substrates with olefin tethers substituted at different positions, for example the α -carbon, *C*-terminus, *N*-terminus, or side-chain. Part of this thesis focuses on the CM of amino acid and peptide-based compounds with side-chain substituted olefin tethers to important biological molecules such as carbohydrates and fatty acids (Chapter 2). Currently there are no general and versatile methods for linking a functionalised amino acid side-chain to a target molecule using CM. The synthesis of amino acid dimers via the same general CM strategy is also addressed in this thesis (Chapter 3). Section 1.6 reviews the application of RCM to amino acid and peptide-based substrates. Part of this thesis describes a general and versatile method for the synthesis of cyclic compounds containing different ring sizes via RCM from acyclic precursors with controllable olefin tether lengths (Chapter 4). This method differs from examples given in section 1.6 in that it involves amino acid side-chain derivatisation via acylation with variable olefin tethers that can be applied to different types of natural amino acids.

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CHAPTER TWO

CROSS METATHESIS

2.1 INTRODUCTION

Modifications to peptides and proteins are important biological events that are involved in a wide variety of functions such as structural, signalling and catalysis. The ability to synthetically modify an amino acid or peptide by covalent attachment of a target molecule is crucial in the development of peptidomimetics and therapeutic agents. Examples that have generated widespread interest include the conjugation of amino acids or peptides to carbohydrates (glycopeptides) or to fatty acids (lipopeptides). The following section details some examples that highlight the importance and synthetic preparation of glyco- and lipopeptides.

2.1.1 Applications and Synthesis of Glycopeptides

Carbohydrates are commonly found conjugated to proteins on cell surfaces, and can play a crucial role in processes such as immune recognition.¹ Glycoproteins and glycopeptides also play key roles in cell-adhesion, protease protection, signalling, protein structure, and inflammation processes.² The degree of glycosylation of a peptide can influence its biological function. For example the glycosylation state in disorders such as rheumatoid arthritis can greatly affect collagen recognition by the immune system.³ Alterations in the glycosylation patterns of surface proteins is evident in cancer as a result of oncogenic transformation.¹ The difference in glycosylation state is also important in the blood group types A and B, that differ only by a slight structural variation at the terminal galactose.

The antibiotics vancomycin **2.1** and teicoplanin **2.2** (Figure 2.1) are important examples of glycopeptides that are used extensively for the treatment of Gram-positive bacterial infections such as *Staphylococcus aureus*.⁴ Vancomycin **2.1** consists of a disaccharide covalently linked to residue 4 in the peptide backbone, while teicoplanin **2.2** consists of three covalently linked monosaccharides and a C₁₀ fatty acyl chain covalently linked through an amide bond to the glucosamine carbohydrate moiety. Both antibiotics consist of a heptapeptide scaffold that has undergone extensive oxidative cross-linking, setting the rigid architecture of the drug scaffold. The increasing number of cases of antibiotic

resistance has led to the construction of semi synthetic derivatives of vancomycin **2.1** (e.g. **1.117**, Scheme **1.25**) and teicoplanin **2.2**.⁵

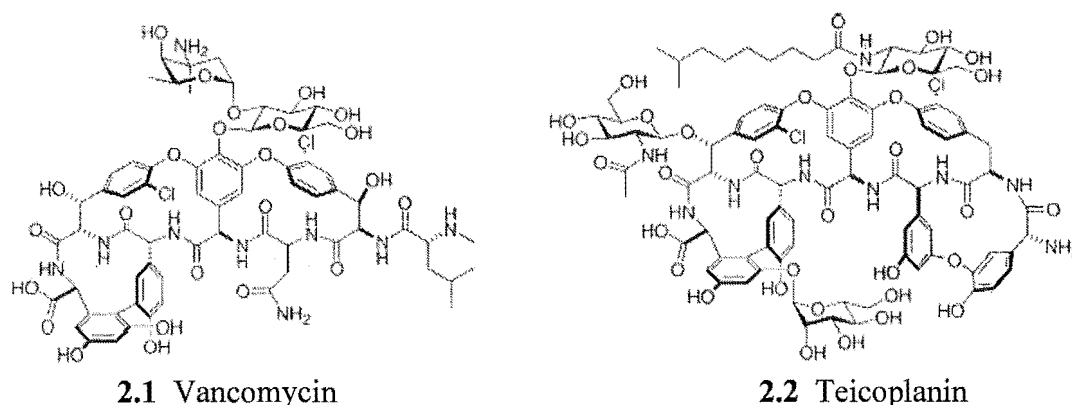


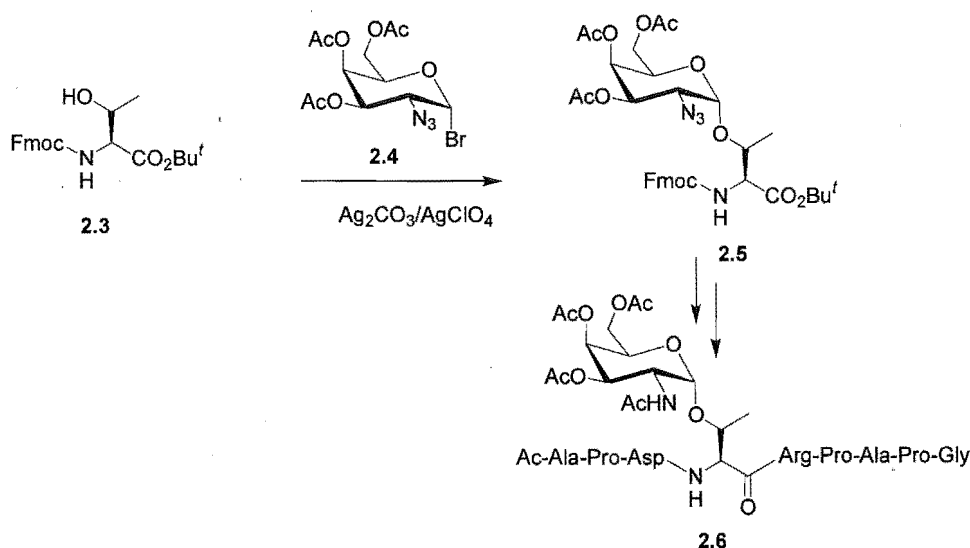
Figure 2.1. Structures of glycopeptide antibiotics vancomycin and teicoplanin.

There is also ongoing interest in the synthesis of smaller glycopeptides, both as direct therapeutics and as mechanistic probes for various medicinal applications.⁶ The chemical synthesis of glycopeptides has advantages over isolation from natural sources, as it allows for the preparation of well-characterised compounds of high purity and reproducibility. An example of glycopeptide synthesis is the construction of α -*O*-glycosyl-linked serine and threonine compounds for the preparation of cancer vaccines.⁷ Synthetic glycopeptides such as these mimic the α -*O*-glycosyl serine moiety that occurs naturally on the surface of tumour cells, and have been conjugated to bovine serum albumin and injected in organisms for the induction of antibodies.⁸ The following section outlines some common synthetic methods used in the key conjugation step of linking an amino acid or peptide to a carbohydrate moiety. Glycopeptides are categorized according to the type of atom of the amino acid that is conjugated to the carbohydrate.

***O*-Linked glycopeptides**

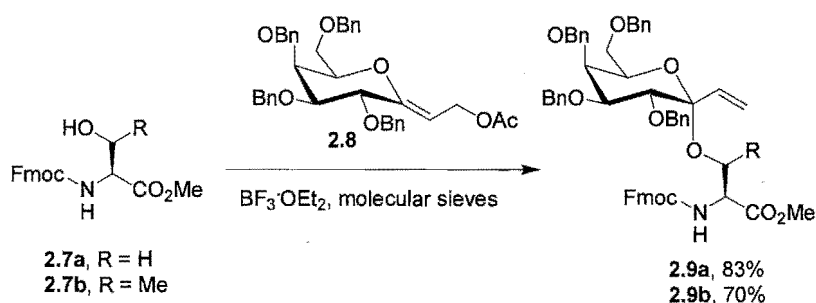
α -*O*-Glycosylated serine and threonine are frequently used in the construction of glycopeptides. They are most commonly synthesised by linking the amino acid side chain alcohol group to the carbohydrate anomeric carbon. For example glycosyl bromide **2.4** has been coupled to threonine **2.3** to afford protected glycoamino acid **2.5** (Scheme **2.1**).⁷

Glycoamino acid building block **2.5** was then incorporated into a peptide sequence to give glycopeptide **2.6**, which is an example of a tumour-associated mucin-type antigen. There are many more examples of other similarly linked threonine or serine glycoamino acids synthesised using slightly modified coupling methods.⁹⁻¹¹



Scheme 2.1.

Glycoamino acids have also been synthesised that contain a quaternary α -anomeric carbon centre with a vinyl group that can then be further elaborated. Glycosylation of either protected serine **2.7a** or threonine **2.7b** with **2.8** in the presence of boron trifluoroetherate proceeds via an allylic rearrangement to give exclusively the α -glycoamino acid products **2.9a** or **2.9b** (Scheme 2.2).¹²



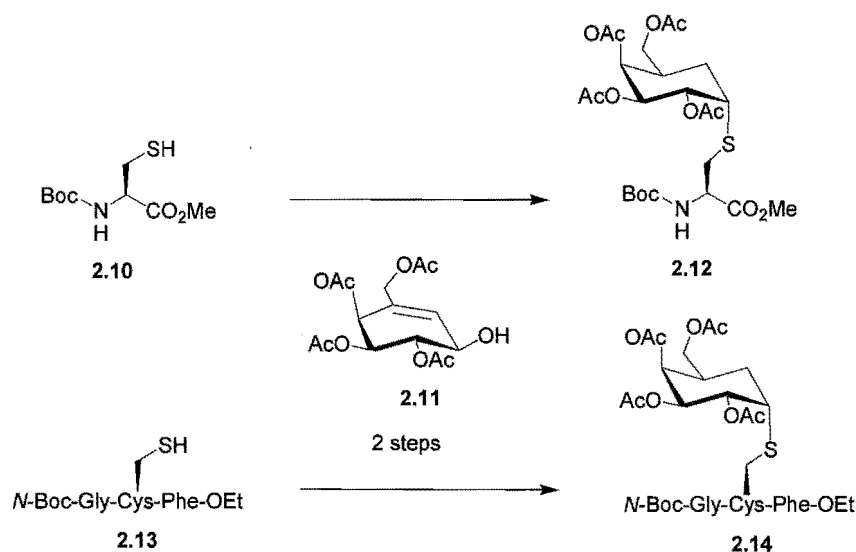
Scheme 2.2.

There are some problems associated with *O*-linked glycoamino acids and peptides despite that fact they are effective therapeutic agents as they resemble the naturally occurring *O*-glycoside linkages so closely. Serine and threonine glycopeptides such as **2.6** are

susceptible to cleavage in biological systems through acid, base, or enzymatic hydrolysis of the *O*-linked tether that is in the same position as in naturally occurring glycopeptides.

***S*-Linked glycopeptides**

Cysteine has been used to construct *S*-glycopeptides by linkage through the side chain thiol group, instead of the alcohol group in *O*-glycopeptides. One example of many is the coupling of activated hexane derivative **2.11** to Boc-*L*-Cys-OMe **2.10** to give *S*-glycoside derivative **2.12** (Scheme 2.3).¹³ Compound **2.12** is an example of a modified amino acid building block that could be incorporated into a peptide. The same methodology was also shown to be applicable to the conjugation of carbohydrate derivative **2.11** to the pre-assembled peptide **2.13**, to give glycopeptide **2.14** (Scheme 2.3).

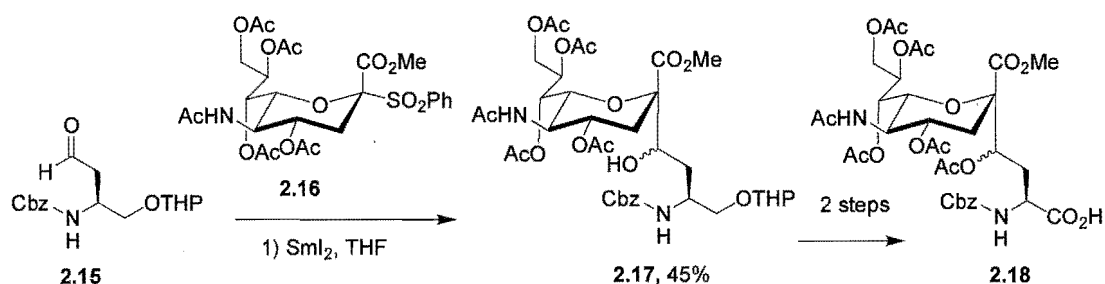


Scheme 2.3.

S-Glycopeptides are potentially unstable in biological systems due to the type and position of linking tether, as is the case for *O*-glycopeptides. This is a potential disadvantage for therapeutic applications, as for example conjugates could be broken down before reaching an intended biological target.

C-Linked glycopeptides

C-Linked glycoamino acids and peptides have been synthesised that are more stable in biological systems towards acid, base, and enzymatic hydrolysis compared to *O*- and *S*-glycopeptides.¹⁴ The coupling reaction of a carbohydrate derivative to an amino acid or peptide to construct a C-glycoside requires the formation of a carbon-carbon bond. An example of a method used to achieve this is the synthesis of a C-linked homoserine conjugate by Linhardt and co-workers.¹⁵ Here glycosyl donor **2.16** was coupled to aldehyde acceptor **2.15** in a samarium diiodide mediated reaction to give α -C-glycoside **2.17** in moderate yield. This was then converted in two steps to C-linked glycoamino acid **2.18** (Scheme 2.4).



Scheme 2.4.

C-Linked glycopeptides synthesised using CM

CM has been used as the key coupling reaction to construct C-linked glycopeptides by a number of research groups in recent years. These systems have been keenly investigated as an effective way to access C-linked glycopeptides that are more biologically stable than their *O*-linked analogues. Some examples of the CM of derivatised amino acids with carbohydrates were outlined in Chapter One, Schemes 1.5-1.9. The application of olefin metathesis to glycobiology has also been reviewed by Overkleeft *et al.*¹⁶

Two different synthetic strategies can be used in the construction of a glycopeptide. A modified carbohydrate can be conjugated to an amino acid to form a glycoamino acid building block, which can then be incorporated into a peptide (e.g. **2.12**, Scheme 2.3, or Schemes 1.5 – 1.9). Alternatively a carbohydrate can be conjugated to an already

assembled peptide (for example **2.14**, Scheme **2.3**). Very recently in January 2005 a patent was published by Davis and Kramer describing the preparation of glycosylated amino acids, proteins, and peptides via CM.¹⁷ Some limitations of this patent in comparison to work carried out in this thesis are outlined in Section **2.4**.

One of the aims of this thesis was to develop a CM method to link a modified carbohydrate to an amino acid or peptide through an alkene side chain tether of variable length. This allowed for control over the alkene tether length, leading to a very versatile general method. Variable tether lengths in peptide conjugates are known to be important in monoclonal antibody production. With this methodology in hand, a protein could potentially be conjugated to an olefin-containing organic molecule, leading to a very powerful and general technique for the synthesis of useful biological conjugates such as glycopeptides (Figure **2.2**). The amino acids used in this project were lysine, serine, and cysteine. These were selected based on previous examples that showed the importance of glyco- and lipoamino acids of these types and because they contained a side-chain functional group that could be easily modified. The use of lysine may be particularly advantageous as it is often found on the hydrophilic surface of proteins, thus side-chain modification and conjugation could be envisioned to have little effect on the overall protein shape that may be crucial for biological activity. A lysine-rich protein (e.g. lysozyme) could potentially be linked to several organic molecules through the many different lysine side chain residues on the protein surface.

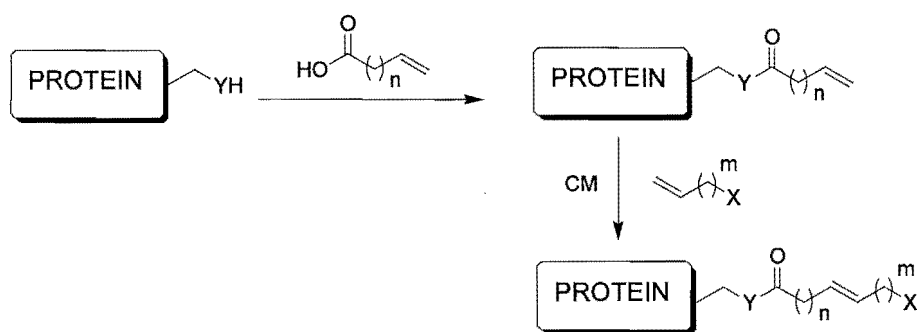


Figure 2.2. General methodology of attachment of target molecule **X** to amino acid side chain **Y** of a protein using CM.

2.1.2 Applications and Synthesis of Lipopeptides

The conjugation of a lipid or fatty acid moiety to an amino acid or peptide can greatly change the physical properties and biological behaviour of the parent peptide. Synthetic lipopeptides can be modified to finely tune desired properties, for example the type and/or chain length of the lipid moiety, the amino acid spacer, and/or the peptide backbone can be varied. Peptides that are unable to cross intestinal mucosal membranes or the blood-brain barrier can be conjugated to fatty acids to increase bioavailability and passive transport membrane permeability.¹⁸ Lipopeptides have been used in the construction of vaccines as membrane delivery agents, and can be very effective at generating a comprehensive immune response. For a comprehensive review of lipopeptide vaccines for diseases such as HIV, malaria, and herpes, see Nesburn *et al.*¹⁹

Lipopeptides have also been used as antibiotics, for example daptomycin **2.19** (Figure 2.3) that is used to treat Gram-positive infections. Daptomycin **2.19** consists of a cyclic peptide with a lipophilic tail that ends with a decanoyl side-chain. Its proposed biological mechanism of action is facilitated by the insertion of the lipophilic tail into the bacteria cell membrane.²⁰ Lipoamino acids have also found use in non medicinal chemistry applications, for example as lubricants, polishes, cosmetics, and as surface weatherproof coatings for ceramics.

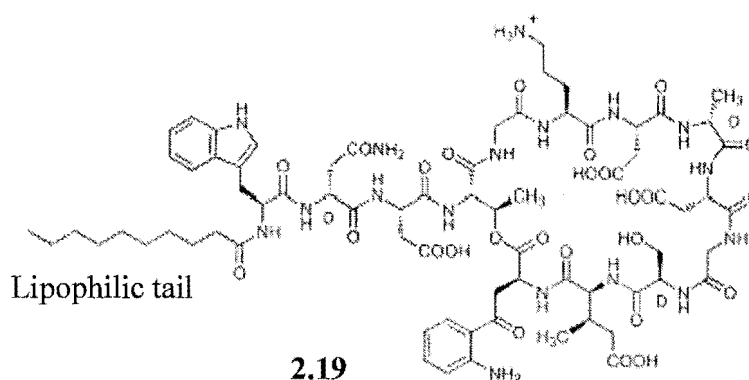
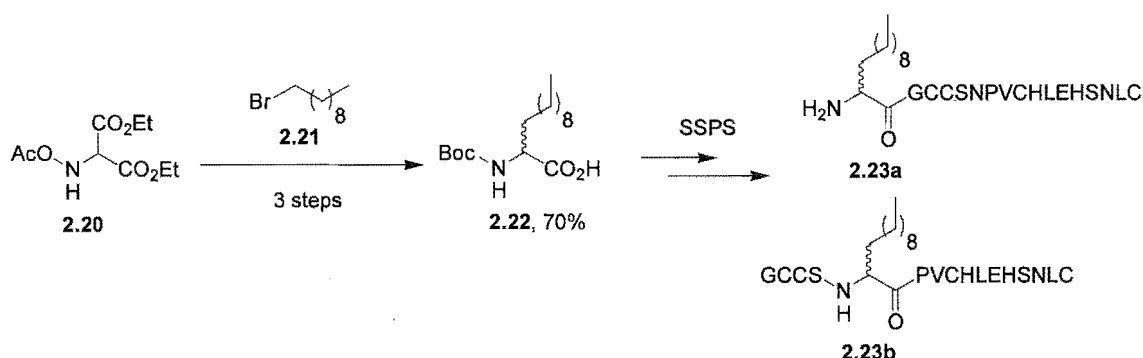


Figure 2.3. Structure of daptomycin lipopeptide antibiotic.

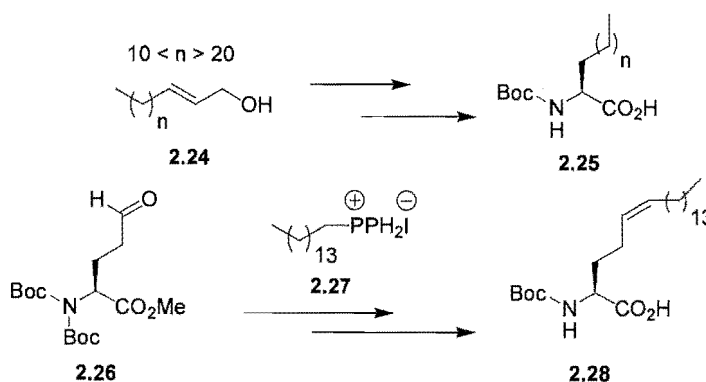
The following section outlines the importance of synthetic lipopeptides and discusses some existing methodologies employed to link an amino acid to a fatty acid derivative.

Fatty acid chains have been directly coupled to the α -carbon of an amino acid in a carbon-carbon bond forming reaction. For example Boc-protected 2-amino-*D,L*-dodecanoic lipoamino acid **2.22** was synthesised as a mixture of enantiomers in three steps from diethyl acetamido malonate **2.20** and 1-bromodecane **2.21** (Scheme 2.5).²¹ Lipoamino acid **2.22** was then incorporated into a peptide at varying positions to give either **2.23a** or **2.23b**. These peptides are cell-permeable analogues of α -conotoxin MII, a potent and highly selective inhibitor of neuronal nicotinic acetylcholine receptors implicated in several disease states including Alzheimer's, Tourette's, and Parkinson's disease.



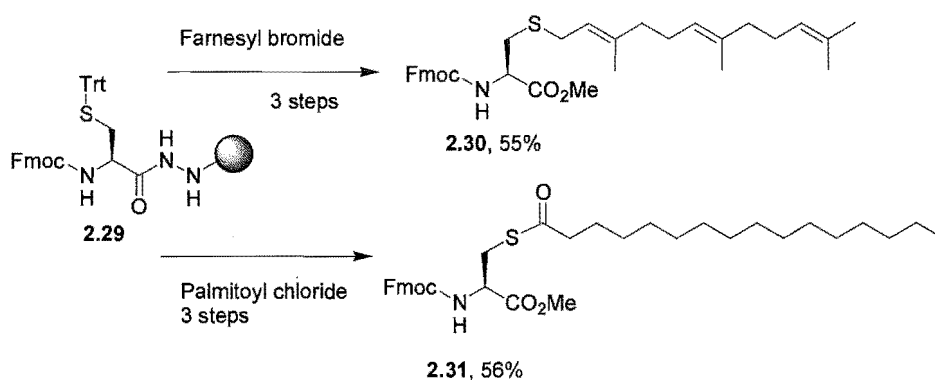
Scheme 2.5.

Enantiomerically pure α -lipoamino acids **2.25** have been synthesised in a stepwise fashion by gradually building up the amino acid functionality from **2.24** (Scheme 2.6).²² Lipoamino acids have also been synthesised using Wittig chemistry, for example the aldehyde of *S*-glutamic acid **2.26** was reacted with ylide **2.27** to give a *Z*-alkene in 92% yield, which was then deprotected to give **2.28** as a pure enantiomer (Scheme 2.6).²³



Scheme 2.6.

Synthetic lipoamino acids have been constructed where the fatty acid moiety is instead linked through the amino acid side chain functionality. For example both isoprenyl and palmitoyl functionalities have been installed as thioether (**2.30**) or thioester (**2.31**) functionalities on the side chain of solid-phase trityl-protected cysteine derivative **2.29** (Scheme 2.7).²⁴ Lipoamino acid building blocks **2.30** and **2.31** were then incorporated into a human Ras protein synthetic analogue and used as a mechanistic probe, as lipidation of Ras proteins is known to be essential for biological activity. There are also examples where various types and lengths of fatty acid chains have been attached to peptides via an *N*-terminal linkage to the peptide backbone.²⁵⁻²⁷



Scheme 2.7.

Part of this thesis, as illustrated in Figure 2.2 where 'X' is a fatty acid, investigates the coupling of amino acids to fatty acid derivatives using a CM coupling reaction. Currently there are no literature reports detailing the application of CM to the construction of lipoamino acids. The synthesis of side-chain cysteine tethered fatty acids described in this thesis has advantages over other cysteine side-chain attachment methods such as shown in Scheme 2.7. These are discussed in this chapter at the end of Section 2.4.

2.2 SYNTHESIS OF MODIFIED AMINO ACIDS

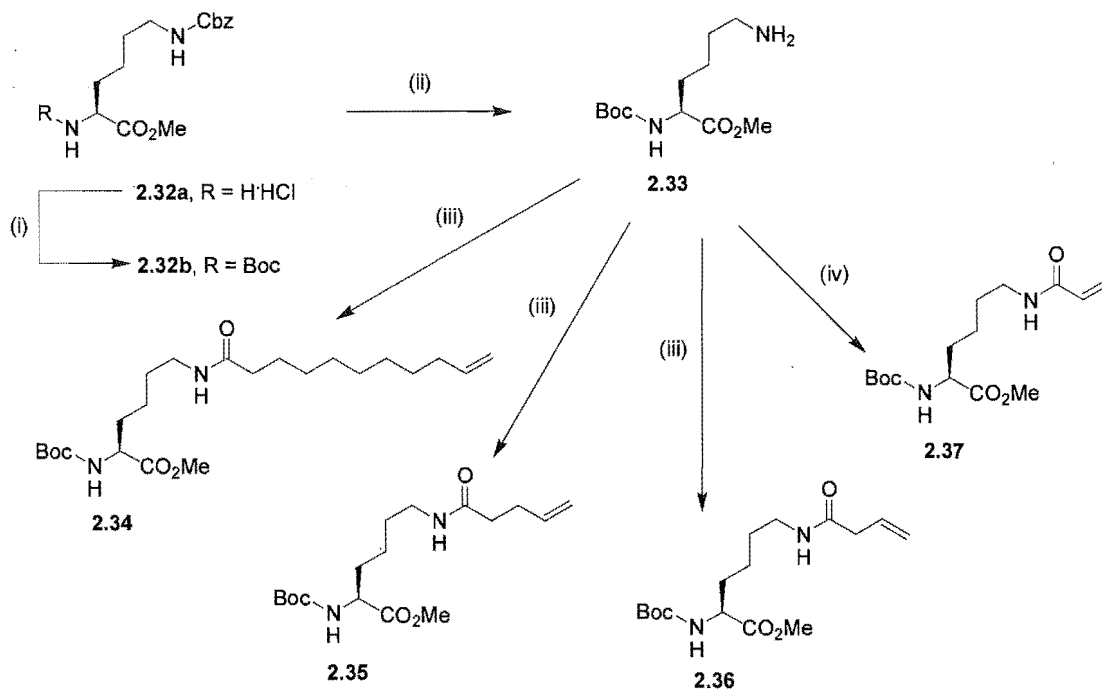
The side-chains of lysine, serine, and cysteine were acylated with an alkene containing carboxylic acid to give amino acid-based substrates that were suitable for CM. This methodology allows for attachment of a variable and controlled alkene tether length. The following section outlines the synthesis of these types of modified amino acids and peptides.

2.2.1 Synthesis of Modified Lysine Compounds

Four different lysine-based CM precursors with different alkene chain lengths were synthesised from common precursor N_α -Boc-*L*-Lys-OMe **2.33** (Scheme 2.8). *N*- And *C*-backbone protected lysine **2.33** was initially purchased from a commercial source and used directly but the quality was dubious, so instead **2.33** was synthesised from commercially available N_ϵ -Cbz-*L*-Lys-OMe hydrochloride salt **2.32a**. Amine salt **2.32a** was Boc-protected using Boc-anhydride to give fully protected **2.32b** in quantitative yield. The Cbz amino-side chain protecting group of **2.32b** was cleaved by hydrogenation over palladium on carbon to afford common precursor **2.33**. N_α -Boc-*L*-Lys-OMe **2.33** was then coupled to 10-decenoic acid using a standard EDCI/HOBt coupling strategy to give long chain N_ϵ -decenoyl lysine **2.34** in 58% yield. 4-Pentenoic acid was reacted with **2.33** under the same coupling conditions to give N_ϵ -pentenoyl lysine **2.35** in a slightly higher yield of 71%. Vinyl acetic acid was also coupled to **2.33** to give N_ϵ -propenoyl lysine **2.36** in 77% yield, as an example of a intermediate tether length.

An N_ϵ -acryloyl lysine analogue was also synthesised as α,β -unsaturated amides have previously been effective in CM reactions.²⁸ Coupling of acrylic acid to common precursor **2.33** was attempted using EDCI/HOBt but the N_ϵ -acryloyl lysine product could not be isolated, perhaps since acrylic acid readily forms polymers that could interfere with the coupling reaction. Acryloyl chloride was instead coupled to **2.33** to afford the desired α,β -unsaturated amide **2.37** in 78% yield (Scheme 2.8). N_ϵ -Acryloyl lysine **2.37** was a sticky oil that was sparingly soluble in DCM. After a few months of storage in a freezer samples of **2.37** resembled hard, treacle-like gum that would not dissolve in DCM or any

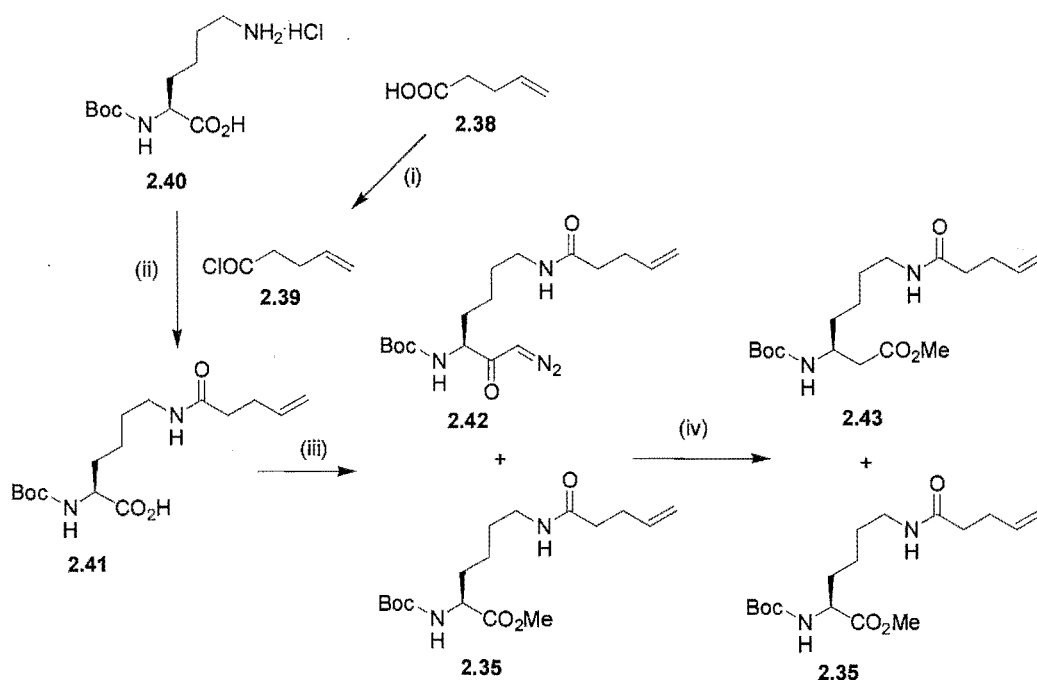
other solvents. It was assumed due to the nature of the *N*-acryloyl group that the compound polymerised over time, therefore samples of **2.37** were prepared within a month of use.



Scheme 2.8. *Reagents and conditions:* (i) Boc_2O , TEA, CH_3CN , rt, 16 h (99%). (ii) 10% Pd/C, H_2 , MeOH, rt, 16 h (98%). (iii) EDCI, HOBt, DIPEA, DCM, rt, 16 h, 10-decanoic acid (**2.34**, 58%) or 4-pentenoic acid (**2.35**, 71%) or vinyl acetic acid (**2.36**, 77%). (iv) DIPEA, acryloyl chloride, DCM, 0°C , then rt, 16 h (78%).

β -Amino acids are a variation of the more abundant α -amino acids, where the amino acid backbone is extended by one sp^3 carbon unit. Research into β -amino acids and their corresponding peptides is of interest for a number of reasons, for example as they are found in a variety of biologically active natural products such as Taxol[®].²⁹ β -Peptides have also shown promising results as therapeutics due to their increased metabolic stability towards protease cleavage and interesting structural properties such as well-defined three-dimensional shapes. A β -amino acid was synthesised (**2.43**) as an example of how a side-chain olefin tethered α -amino acid could be converted into a β -amino acid framework.

The synthesis of β -amino acid **2.43** began with the preparation of key precursor **2.41** (Scheme 2.9). 4-Pentenoic acid **2.38** was reacted with oxalyl chloride in dry ether with a catalytic amount of DMF to give 4-pentenoyl chloride **2.39**. Commercially available Boc-*L*-lysine hydrochloric salt **2.40** was acylated at the side chain amine with 4-pentenoyl chloride **2.39** in aqueous conditions to afford N_α -Boc- N_ϵ -pentenoyl- α -lysine **2.41** in 68% yield. α -Amino acid **2.41** was converted to the N_α -Boc- N_ϵ -pentenoyl- β -lysine methyl ester **2.43** in two steps. Carboxylic acid **2.41** was treated with TEA and ethyl chloroformate to form the mixed anhydride, followed by dropwise addition of diazomethane in ether to give diazoketone **2.42**. This transformation is known as the Arndt-Eistert synthesis.^{30,31} Diazoketone **2.42** then underwent a Wolff rearrangement in the presence of silver benzoate and methanol to give β -lysine methyl ester **2.43**. The Wolff rearrangement mechanism is believed to involve the loss of nitrogen gas to give a carboanion that rearranges via intramolecular attack to give a ketene, which then reacts with methanol to give **2.43**.

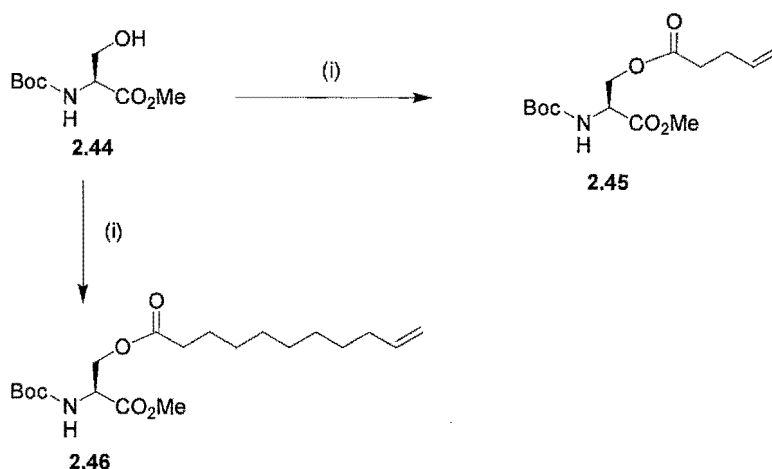


Scheme 2.9. Reagents and conditions: (i) Oxalyl chloride, DMF, ether, 0°C then rt, 16 h (81% after distillation). (ii) **2.39**, NaOH, CH₃CN, rt, 2 h (68%). (iii) TEA, ClCO₂Et, THF, -15°C, 15 min, warm to 0°C, then CH₂N₂/ether, warm to rt, 3 h. (iv) AgOBn, TEA, MeOH, -25°C, 3 h (73% yield of **2.43** over 2 steps).

The crude reaction mixture following Wolff rearrangement gave a mixture of β -lysine methyl ester **2.43** and α -lysine methyl ester **2.35** (Scheme 2.9). The formation of **2.35** is presumably due to the non-quantitative reaction of ethyl chloroformate with **2.41** to form the mixed anhydride in the preceding Arndt-Eistert step. Any unreacted carboxylic acid **2.41** would be methylated upon treatment with diazomethane to give α -lysine methyl ester **2.35**. Diazoketone **2.42** was not purified, so presumably both **2.42** and **2.35** were present in the Wolff rearrangement step, with only diazoketone **2.42** reacting to give **2.43**. A pure sample of β -amino acid **2.43** was eventually obtained from the crude mixture also containing **2.35** by purification using silica gel-based column chromatography. This was difficult as the compounds **2.43** and **2.35** have very similar polarities as they differ by only one methylene group.

2.2.2 Synthesis of Modified Serine and Cysteine Compounds

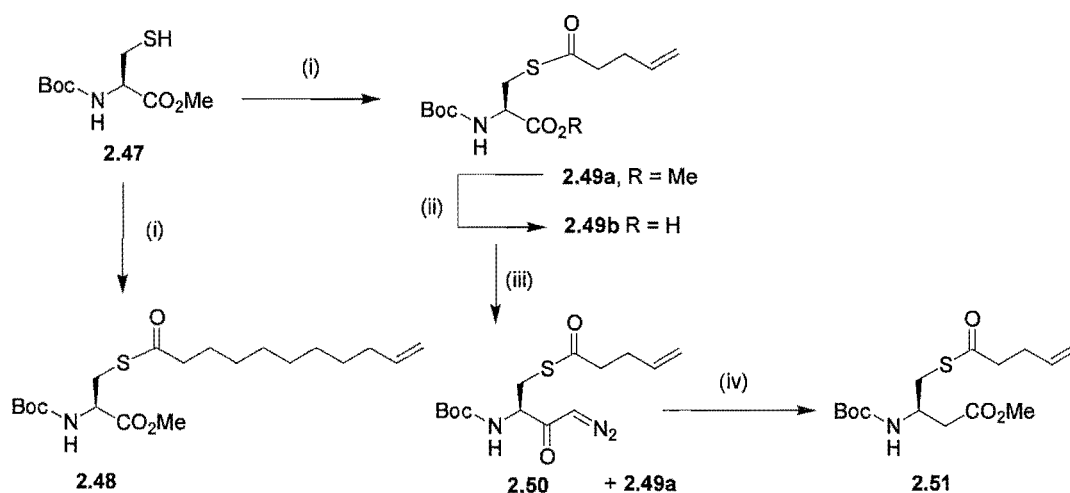
Two modified serine-based compounds were synthesised with different side-chain tether lengths. Commercially available Boc-*L*-Ser-OMe **2.44** was acylated with 4-pentenoic acid in the presence of EDCI/DMAP to afford *O*-pentenoyl serine **2.45** in 89% yield (Scheme 2.10). The same coupling conditions were used to attach 10-decenoic acid to **2.44**, to give *O*-decenoyl serine **2.46** in 84% yield as an example with an extended tether.



Scheme 2.10. *Reagents and conditions:* (i) EDCI, DMAP, DCM, rt, 16 h, 4-pentenoic acid (**2.45** 89%) or 10-decenoic acid (**2.46**, 84%).

The amino acid cysteine was also modified to contain an olefin tether, which was installed by reaction of the side-chain thiol group. Boc-*L*-Cys-OMe **2.47** was coupled to 10-decenoic acid using BOP-Cl to give *S*-decenoyl cysteine **2.48** in 82% yield (Scheme 2.11). Cysteine **2.47** was also coupled to 4-pentenoic acid to afford fully protected *S*-pentenoyl cysteine **2.49a** in slightly a higher yield of 93%.

S-Pentenoyl- β -cysteine cysteine **2.51** that contained a side-chain substituted olefin tether suitable for CM elaboration was also prepared. α -Cysteine **2.49a** was treated with lithium hydroxide in a THF/water mixture to hydrolyse the methyl ester and give carboxylic acid **2.49b** (Scheme 2.11). The stability of the pentenoyl thioester group of **2.49a** under these conditions was of concern, but subsequent Arndt-Eistert synthesis proceeded in high yield, indicating that cleavage of the thio ester of **2.49a** was minimal. Treatment of carboxylic acid **2.49b** with TEA and ethyl chloroformate, followed by addition of diazomethane afforded a mixture of diazoketone **2.50** and a small amount of α -cysteine methyl ester **2.49a**. Because of the problems encountered isolating β -lysine **2.43** from α -lysine **2.35** (Scheme 2.9), diazoketone **2.50** was purified by silica gel-based chromatography as it was anticipated that separation of a mixture of **2.50** and **2.49a** would be easier than a mixture of **2.51** and **2.49a** following Wolff rearrangement.



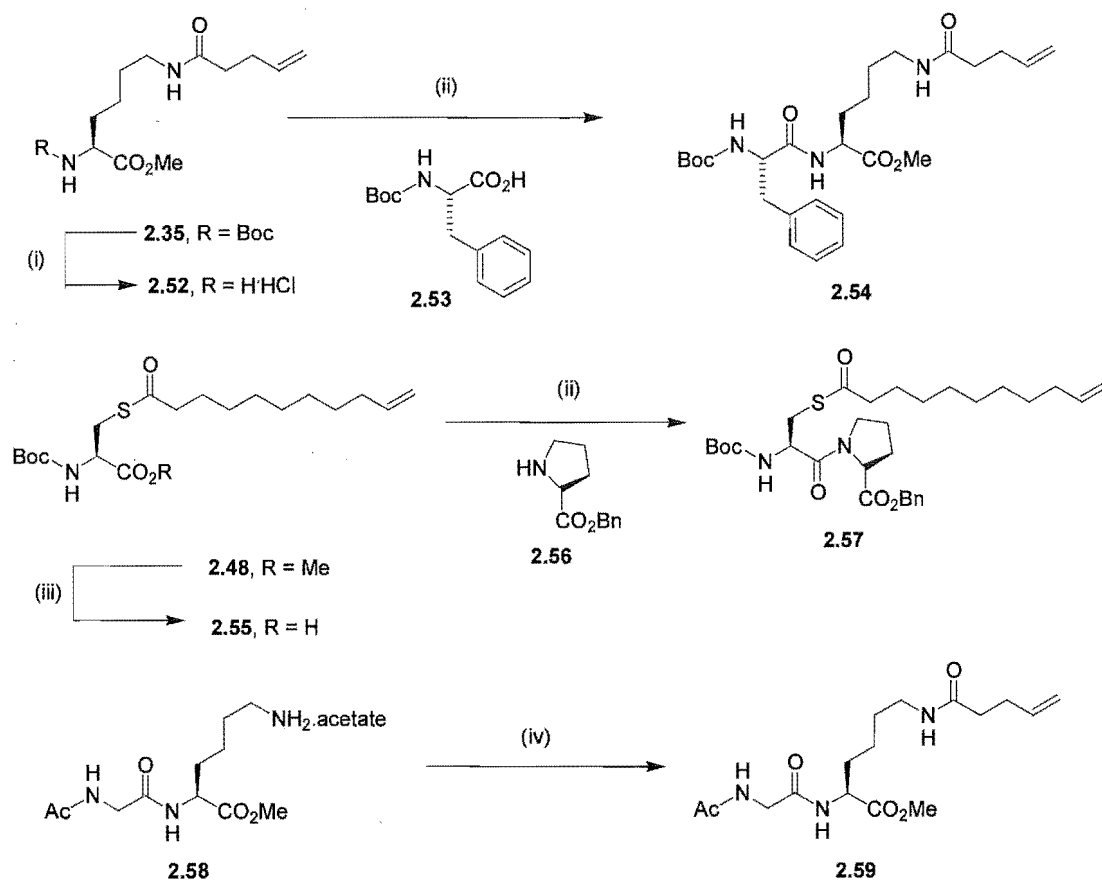
Scheme 2.11. *Reagents and conditions:* (i) BOP-Cl, TEA, DCM, 0°C then rt, 4 h, 10-decenoic acid (**2.48**, 82%) or 4-pentenoic acid (**2.49a**, 93%). (ii) LiOH_{aq} (0.2M), THF, 0°C, 30 min. (iii) TEA, ClCO₂Et, THF, -15°C 15 min, warm to 0°C, then CH₂N₂/ether, warm to rt, 3 h (82% yield of **2.50** over 2 steps). (iv) AgOBn, TEA, MeOH, -25°C, 3 h (51%).

Diazoketone **2.50** was treated with silver benzoate, TEA, and methanol to give β -cysteine methyl ester **2.51** in 51%. Synthesis of the corresponding β -serine was not carried out, since it was considered that the general method of β -amino acid formation from olefinic side chain-derivatised α -amino acids had adequately been demonstrated.

2.2.3 Synthesis of Modified Dipeptide Compounds

As an extension of amino acid-based CM precursors in sections 2.2.1 and 2.2.2, dipeptides were synthesised in a step towards using larger peptides and eventually proteins for these types of studies. *N*_ε-Pentenoyl lysine **2.35** was Boc-protected by bubbling hydrochloric gas through an ethyl acetate solution to give hydrochloride salt **2.52** (Scheme 2.12). Boc-*L*-Phe-OH **2.53** was coupled to hydrochloric salt **2.52** using EDCI/HOBt coupling conditions to afford *N*_ε-pentenoyl-Lys-Phe dipeptide **2.54** in 63% yield. An example of a dipeptide containing an *S*-acyl olefin linkage was also synthesised. The methyl ester of **2.48** was hydrolysed with aqueous lithium hydroxide to give carboxylic acid **2.55**, which was then coupled to proline benzyl ester **2.56** using EDCI/HOBt to afford *S*-decenoyl substituted dipeptide **2.57** in 72% yield.

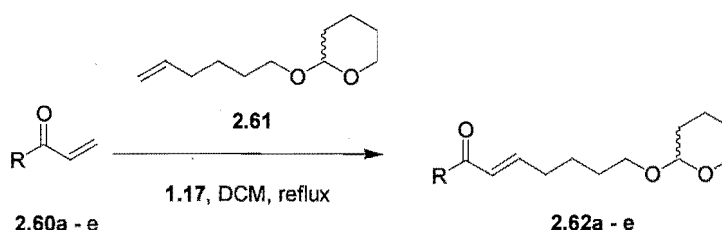
The dipeptides **2.54** and **2.57** were synthesised by the peptide coupling of an amino acid pre-installed with an olefin tether to a second amino acid. An example involving the substitution of an olefin tether to a pre-assembled dipeptide was demonstrated with the synthesis of dipeptide **2.59**. Commercially available *N*-acetyl-*L*-Gly-*L*-Lys-OMe **2.58** was reacted with 4-pentenoic acid in the presence of EDCI/HOBt to give *N*_ε-pentenoyl peptide **2.59** in excellent yield (Scheme 2.12). Dipeptide **2.59** was isolated as a fine, white solid that was very sparingly soluble in DCM, likely due to the polar acetate protecting group. Attempts to synthesise and isolate the corresponding *N*-acryloyl α,β -unsaturated derivative by reaction of **2.58** with either acrylic acid or acryloyl chloride were unsuccessful, presumably due to insolubility issues.



Scheme 2.12. *Reagents and conditions:* (i) HCl gas, ethyl acetate, rt, 4 h (99%). (ii) EDCI, HOBt, DIPEA, DCM, rt, 16 h, Boc-*L*-Phe-OH (**2.54**, 63%) or *L*-Pro-OBn (**2.57**, 72% over 2 steps). (iii) LiOH_{aq} (0.2M), THF, 0°C, 30 min. (iv) EDCI, HOBt, DIPEA, DCM, 4-pentenoic acid, rt, 16 h (93%).

2.3 MODEL STUDY: CONJUGATION OF AMINO ACIDS TO TERMINAL ALKENES

A model study was carried out in which terminal protected alcohols were coupled to derivatised amino acids synthesised in Section 2.2. This was undertaken to ascertain the effectiveness of CM with these amino acid substrates before conjugation to the more complex carbohydrates or fatty acids was attempted. The choice of coupling partner was based upon an earlier study by Grubbs and colleagues²⁸ that showed CM between protected terminal alkene **2.61** and α,β -unsaturated amides **2.60a-e** proceeded effectively to give products **2.62a-e** (Scheme 2.13).

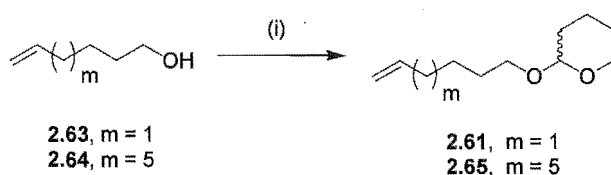


Alkene substrate	R	Product	CM yield
2.60a		2.62a	80%
2.60b		2.62b	89%
2.60c		2.62c	90%
2.60d		2.62d	97%
2.60e		2.62e	100%

Scheme 2.13.

2.3.1 Synthesis of protected terminal alkene

The required protected terminal alkenes were prepared as outlined in Scheme 2.14. Hexen-1-ol **2.63** was treated with dihydropyran in the presence of *p*-toluene sulfonic acid monohydrate to give protected terminal alkene **2.61** in quantitative yield. Similarly decen-1-ol **2.64** was converted into protected **2.65** in 90% yield. Both **2.61** and **2.65** were isolated after silica gel-based column chromatography as racemic mixtures.



Scheme 2.14. *Reagents and conditions:* (i) Dihydropyran, *p*-TsOH, DCM, 18 h (**2.61**, 99%), (**2.65**, 90%).

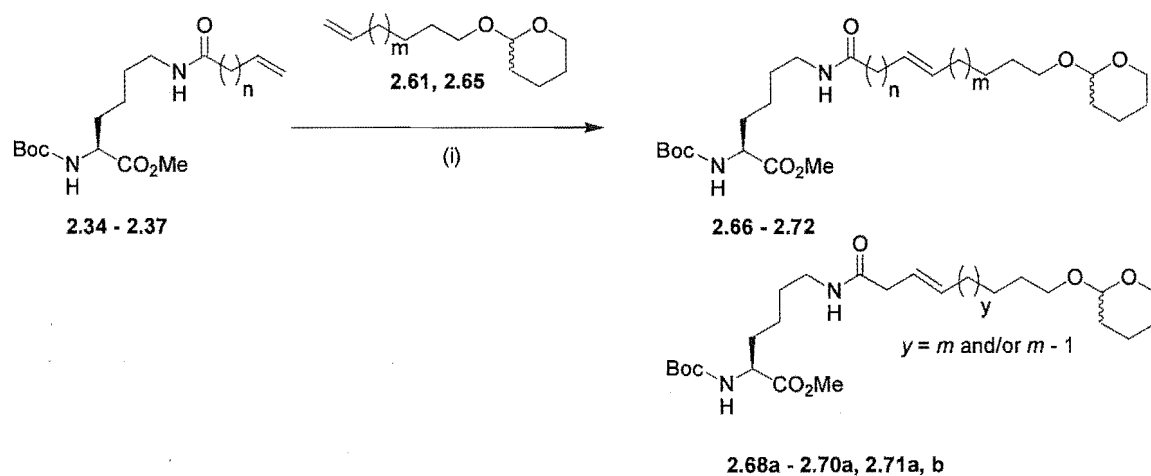
2.3.2 Cross metathesis reactions

CM reactions between modified amino acids (**2.34-2.37**, **2.48**, **2.54**) and terminal alkenes (**2.61**, **2.65**) were carried out in refluxing DCM using 20 mol% of Grubbs second generation catalyst **1.17** (Scheme 2.15). A flow of nitrogen was used to remove the ethylene gas side product, with DCM periodically added due to evaporation in order to maintain the approximate starting substrate concentration (refer to section 1.4, Chapter 1). One equivalent of the amino acid coupling partner and three equivalents of the other terminal alkene coupling partner were used to optimise the cross-coupled product yield (see Chapter 1, Figure 1.5). The protected alcohol terminal alkene CM homodimer was also isolated in all cases. CM reactions were worked up by adding fifty equivalents of DMSO (relative to catalyst) to assist the removal of coloured ruthenium by-products.³²

Despite several purification attempts the CM products shown in Scheme 2.15 were isolated as an inseparable mixture containing traces of isomers. These trace isomers were only assigned if distinguishable peaks were observed in ^1H NMR spectra that could be unequivocally assigned to a particular isomer structure. This was possible for isomer products **2.68a** – **2.70a**, **2.71a** – **b**, and **2.74a** (Scheme 2.15) that contained an α,γ -unsaturated amide functionality, formed as a result of chain contraction and/or double bond migration as discussed in section 2.3.3. The precise structures of these isomers in relation to the chain length ' y ' was not determined, also as discussed in section 2.3.3. Traces of compounds other than shown in Scheme 2.15 were likely also produced due to bond migration, but were not assigned due to the lack of a distinctive resonance in ^1H NMR spectra. All CM products shown in Scheme 2.15 were assigned as *E*, reasons for which are discussed later in this section. The yields of CM reactions were calculated using the total mass of the isolated cross-coupled sample following silica gel-based column chromatography and assumed the mass of the major CM product.

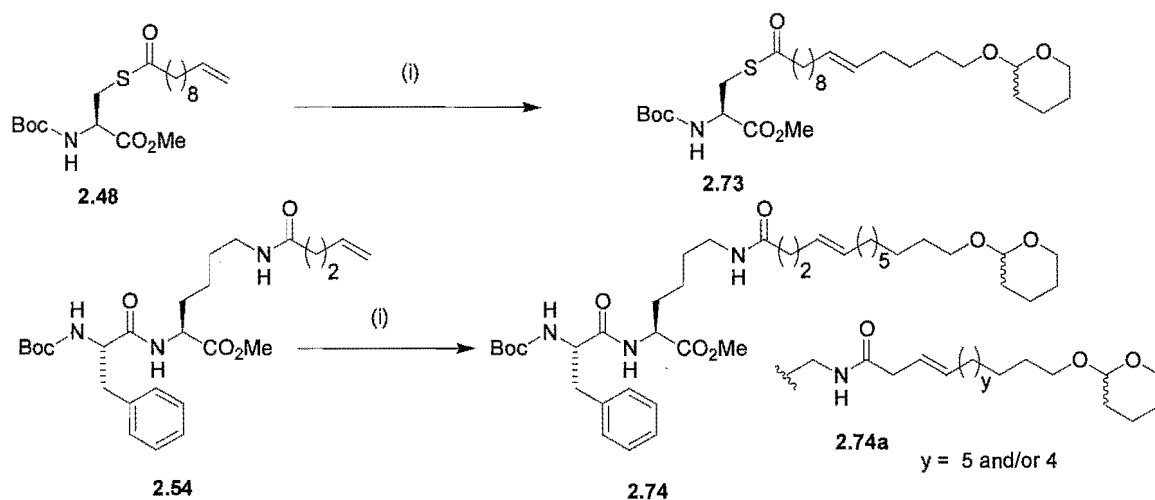
α,β -Unsaturated amide **2.37** was coupled to hexen-1-ol **2.61** and decen-1-ol **2.65** terminal alkenes in separate reactions to give CM products **2.66** (91%, entry 1) and **2.67** (75%, entry 2) (Scheme 2.15). *N*-Propenoyl lysine **2.36** was coupled to terminal alkene **2.61** to afford a mixture containing major CM product **2.68** and isomer **2.68a** (3:1 **2.68**/**2.68a**, 57%, entry 3). Isomer **2.68a** was presumed to be formed due to chain contraction (i.e. $y = m-1$) as the major CM product **2.68** ($y = m$) also contains an α,γ -unsaturated amide. CM between *N*-propenoyl lysine **2.36** and terminal alkene **2.65** was carried out to give a mixture of **2.69** and **2.69a** (3:1 **2.69**/**2.69a**, 56%, entry 4) in slightly lower yield. Relative amounts of isomers were determined using ^1H NMR integral ratios.

N-Pentenoyl lysine **2.35** was reacted with **2.61** to give **2.70** and traces of **2.70a** (6:1 **2.70**/**2.70a**, 54%, entry 5). *N*-Pentenoyl lysine **2.35** was also coupled to terminal alkene **2.65** to afford major CM product **2.71** along with traces of **2.71a** and **2.71b** (23:4:1 **2.71**/**2.71a**/**2.71b**, 51%, entry 6), which were formed as a result of ring contraction and/or double bond migration. The longer chain *N*-decenoyl lysine **2.34** was coupled to protected hexen-1-ol **2.61** to afford CM product **2.72** (67% yield, entry 7).



Entry	n	Amino acid	m	Coupling partner	CM Product	Yield	α,γ -unsat. amide isomer [#]	Ratio*
1	0	2.37	1	2.61	2.66	91%	-	-
2	0	2.37	5	2.65	2.67	75%	-	-
3	1	2.36	1	2.61	2.68	57%	2.68a	3:1
4	1	2.36	5	2.65	2.69	56%	2.69a	3:1
5	2	2.35	1	2.61	2.70	54%	2.70a	6:1
6	2	2.35	5	2.65	2.71	51%	2.71a, b	23:4:1
7	8	2.34	1	2.61	2.72	67%	-	-

* Ratio of CM product/ α,γ -unsaturated isomer. [#] Not isolated, present as a mixture.



Scheme 2.15. Reagents and conditions: (i) Grubbs second generation catalyst 1.17 (20 mol%), 2.61 or 2.65 (3 equiv), DCM, argon flow, reflux 6 h, then DMSO (50 equiv relative to 1.17), rt, 12 h (2.73, 36%), (2.74/2.74a 5:1, 56%).

Analysis of the table shown in Scheme 2.15 reveals that *N*-acryloyl products **2.66** (entry 1) and **2.67** (entry 2) were isolated in the highest yield (compare entries 1 and 3, 2 and 4). An explanation for the high CM yields when using α,β -unsaturated amide **2.37** could be due to its olefin Type, as defined in Table 1.1, Chapter One. α,β -Unsaturated amide **2.37** is defined as a Type II olefin, while protected alcohols **2.61** and **2.65** and amino acid-based substrates **2.34** – **2.36** are more characteristic of Type I olefins. Chatterjee and Grubbs have suggested that CM between a Type I (e.g. **2.61**, **2.65**) and Type II (e.g. **2.37**) olefin is selective and gives the cross-coupled product in higher yield, compared to CM between two Type I olefins that gives a lower, statistical yield of the cross-coupled product.³³ The higher yields of CM products using substrate **2.37** compared to **2.34** – **2.36** was also in line with a study by Grubbs *et al*²⁸ that showed α,β -unsaturated olefins are particularly effective CM substrates (Scheme 2.13).

The longer *N*_ε-decenoyl olefin tether length of **2.34** (entry 7) would also appear to be favourable for CM, as although **2.34** gave lower CM yields compared to α,β -unsaturated amide **2.37** (entries 1, 2), it gave higher CM yields compared to both *N*_ε-pentenoyl lysine **2.35** (entries 5, 6) and *N*_ε-propenoyl lysine **2.36** (entries 3,4). This may have been due to reduced steric clashing between the catalyst and amino acid backbone for the longer olefin tether length of **2.34**, compared to the shorter side-chain tethers of lysine **2.35** and **2.34**. This trend was in agreement with other studies, for example as shown in Scheme 1.1, Chapter 1, where amino acid α -carbon substituted olefin tethers of longer lengths gave higher CM yields compared to shorter olefin tether lengths.³⁴ Longer length olefin tethers substituted at the *N*-terminus position of amino acids have also been shown to give higher CM yields than shorter olefin tether lengths (Scheme 1.13, Chapter 1).³⁵ Comparison of the CM product yields for terminal alkene coupling partners **2.61** and **2.65** reveals that **2.61** gave slightly higher but comparable yields when coupled to analogous modified amino acid substrates (compare entries 1 and 2, 3 and 4, 5 and 6).

S-Decenoyl cysteine **2.48** was coupled to terminal alkene **2.61** to give CM product **2.73** in 36% yield (Scheme 2.15), which is significantly lower than was observed for the corresponding *N*_ε-decenoyl lysine **2.72** (67%, entry 7). An ¹H NMR spectrum of each of the crude CM products **2.72** and **2.73** showed only evidence of HRC=CRH coupled products, and in both cases the only compounds isolated following silica gel-based

chromatography were the CM product (**2.72** or **2.73**) and the terminal alkene homodimer. This suggested that in each case CM was equally as efficient despite the difference in CM yield. Therefore the difference in product yield was likely due to isolation issues, although it would be anticipated that the opposite would apply, i.e. that the more non-polar cysteine-based **2.73** would be more easily purified using silica gel-based chromatography than the more polar lysine-based **2.72**. CM reactions to give **2.72** and **2.73** were repeated, and gave the same difference in CM yields between the two products.

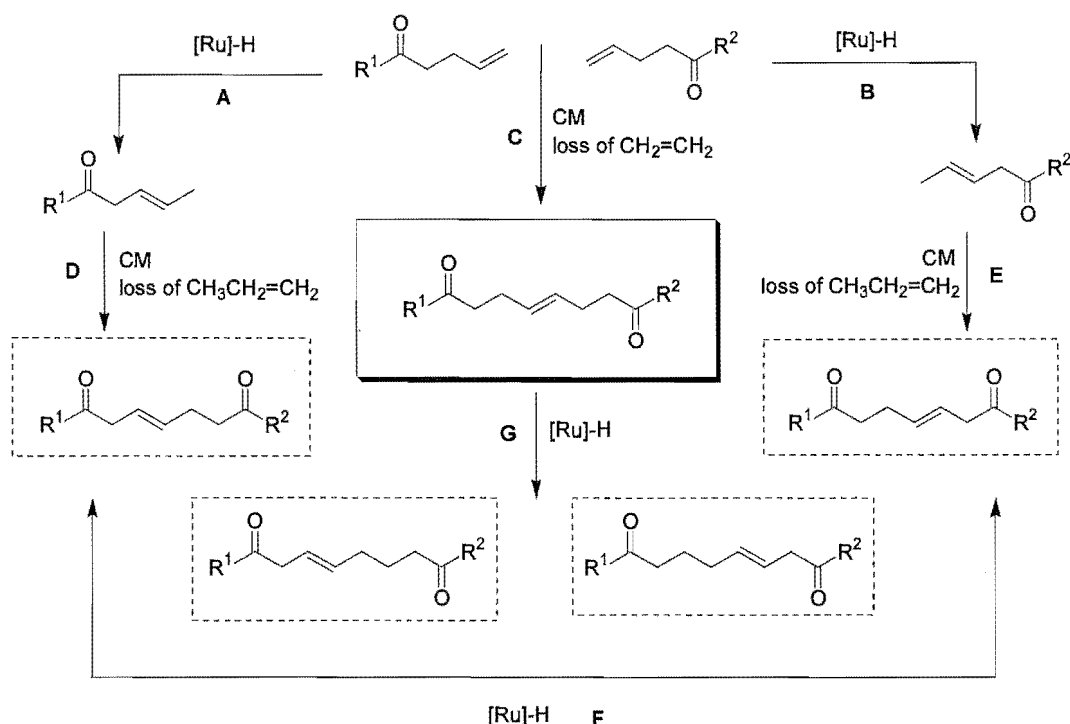
Dipeptide **2.54** was coupled to the protected terminal alkene **2.65** under CM conditions to give a 5:1 mixture of CM product **2.74** and isomer **2.74a** respectively in 56% yield (Scheme **2.15**). This was a promising result as *N*-pentenoyl single amino acid CM product **2.71** was produced in a comparable yield of 51% (entry 6), indicating that the method is equally applicable to larger molecular architectures.

It is important to note that all CM products shown in Scheme **2.15** are presumed to be a 1:1 mixture of epimers since the terminal alkenes **2.61** and **2.65** are racemic. Surprisingly the ^1H and ^{13}C NMR spectra of CM products shown in Scheme **2.15** did not reveal any evidence of diastereoisomers, as only a single resonance at approximately δ_{H} 4.5 ppm and δ_{C} 99 ppm for the OCHO tetrahydropyran atoms, and one set of peaks at δ_{C} 62 and 67 ppm for the OCH₂ atoms was observed. Presumably this was because the OCHO atoms in the diastereoisomers were in chemically equivalent environments, perhaps due to the distance away from the chiral amino acid stereocentre.

The configuration of the double bond formed during olefin metathesis can be *E* or *Z*. Studies have shown that olefin metathesis using catalyst **1.17** is under thermodynamic control, and preferentially gives the most thermodynamically stable double bond configuration in excess.^{36,37} The CM products shown in Scheme **2.15** were all assigned as *E*. This was determined on the basis of the vicinal coupling constant across the double bond for **2.66** – **2.70**, and **2.74**, and where the measurement of this coupling constant was not possible the CM product was assumed *E* for thermodynamic reasons. It was possible that traces of the corresponding *Z* isomers were present in CM products *E*-**2.66** – **2.74**, however distinguishable peaks were not observed in the ^1H and ^{13}C NMR spectra of **2.66** – **2.74** that could be assigned as such.

2.3.3 Double bond migration during cross metathesis

Double bond migration can occur in metathesis substrates prior to CM coupling and/or in the coupled product following CM, which gives rise to isomers which are chain contracted and/or contain a double bond in a different position compared to the major CM product. Migration is believed to be catalysed by a ruthenium hydride species produced as a result of catalyst breakdown over time.³⁸ Ruthenium hydride species are in fact used in organic synthesis for intentional double bond isomerization reactions.³⁹



Scheme 2.16. Example of isomers that could be produced due to ruthenium hydride catalysed double bond migration during CM between two pentenyl substrates.

As an example, Scheme 2.16 illustrates double bond migrated isomers that could be formed during CM between two pentenyl alkenes. CM without double bond migration (Step C) gives the expected cross-coupled product. Depending on the rate of this reaction, some ruthenium hydride species may form *in situ* that could catalyse double bond migration in either CM substrate (Step A or B), the products of which could then undergo CM (Step D or E) to produce chain-contracted products.⁴⁰ These contracted products would in fact also be convertible after CM had occurred via double bond migration (Step

F). Double bond migration is also possible after CM (Step G),⁴¹ and gives rise to CM products of the same chain length as the CM product in Step C but containing a double bond in a different position.

Double bond migrated compounds **2.68a-2.70a**, **2.71a-b**, and **2.74a** were assigned as such by the observation of a small doublet at approximately δ_H 2.9 – 3.0 ppm in the 1H NMR spectra of the respective CM products **2.68-2.71** and **2.74** that did not correspond to the expected non-double bond migrated CM product. This doublet is due to the α,γ -unsaturated amide of the double bond migrated isomers, confirmed by COSY spectrum that revealed coupling of this doublet to a small resonance at approximately δ_H 5.3 – 5.5 ppm in the olefinic proton region.

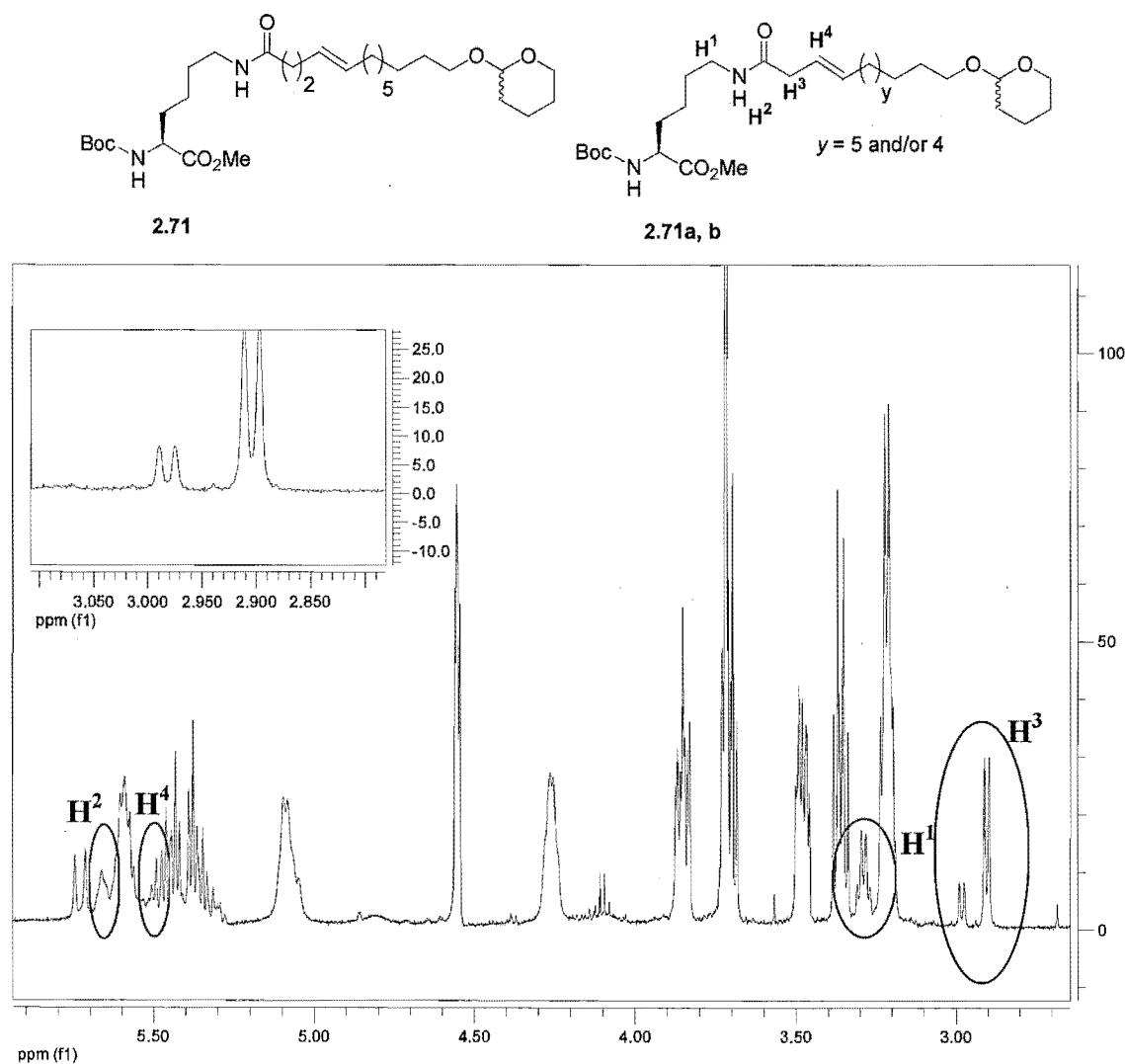


Figure 2.4. 1H NMR ($CDCl_3$, 500 MHz) of **2.71**, with resonances due to double bond migrated α,γ -unsaturated isomers **2.71a - b** circled.

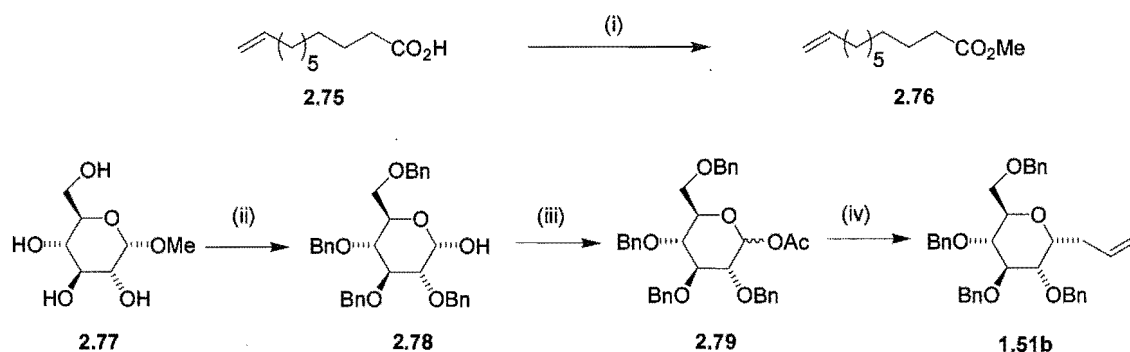
Figure 2.4 shows the ^1H NMR spectrum of **2.71**, which contains two double bond migrated isomers (**2.71a** and **2.71b**), assigned by the presence of two distinguishable doublets between δ_{H} 2.9 - 3 ppm. A small peak in the HRMS corresponding to a chain contracted product containing one less methylene unit compared to the major CM product was observed in all products shown in Scheme 2.15 apart from **2.66** and **2.67**. These traces of chain contracted products could not be purified from the reaction mixture, and as such their entire structure was not assigned. For example although **2.68a-2.70a**, **2.71a-b**, and **2.74a** can be identified as double bond migrated isomers by the presence of a doublet at approximately δ_{H} 2.9 ppm, the entire structure, or in other words the 'y' in structures **2.68a-2.70a**, **2.71a-b**, and **2.74a**, was not determined. Double bond migrated products can be chain contracted compounds (e.g. step **D** or **E**, Scheme 2.16) or a result of double bond migration after CM (e.g. step **G**, Scheme 2.16).

This model study demonstrated that the side-chain modified amino acids synthesised in Section 2.2 could be successfully coupled in CM reactions to alcohol-protected terminal alkenes. This then set the stage for conjugation of the amino acid substrates to biologically relevant molecules such as modified sugars and fatty acids.

2.4 CROSS METATHESIS OF MODIFIED AMINO ACIDS TO A FATTY ACID OR CARBOHYDRATE COMPOUND

2.4.1 Synthesis of protected fatty acid and carbohydrate

Fatty acid and carbohydrate coupling partners containing an olefin were synthesised for use in CM reactions. 10-Decenoic fatty acid **2.75** was esterified using Amberlyst 15 ion-exchange resin to give fatty acid methyl ester **2.76** as a pure colourless liquid (Scheme 2.17). Carboxylic acid protection of **2.75** was carried out as in some CM studies with catalyst **1.17** olefinic compounds containing this functional group were not tolerated.³⁴



Scheme 2.17. *Reagents and conditions:* (i) Amberlyst 15 ion-exchange resin, MeOH, rt, 7 h (75%). (ii) Benzyl chloride, KOH, dioxane, reflux, 3 h (30%). (iii) Pyridine, acetic anhydride, rt, 16 h (97%). (iv) Allyltrimethylsilane, boron trifluoroetherate, CH₃CN, 4°C, 24 h (50%).

The carbohydrate scaffold **1.51b** based on tetra-benzyl α -D-glucopyranose was also prepared (see Chapter 1, Scheme 1.7).⁴² Commercially available methyl α -D-glucopyranoside **2.77** was protected by reaction with benzyl chloride in alkaline conditions that also cleaved the methyl group of **2.77** to give **2.78** in a notoriously low yielding reaction. However the benzyl protecting group has the advantage that it imparts improved solubility in DCM, the solvent used for CM reactions with catalyst **1.17**. The anomeric hydroxyl group of **2.78** was acylated on treatment with acetic anhydride and pyridine to afford acetyl **2.79** as a mixture of anomers at the anomeric carbon centre. Treatment of acetyl **2.79** with allyltrimethylsilane in acetonitrile in the presence of boron

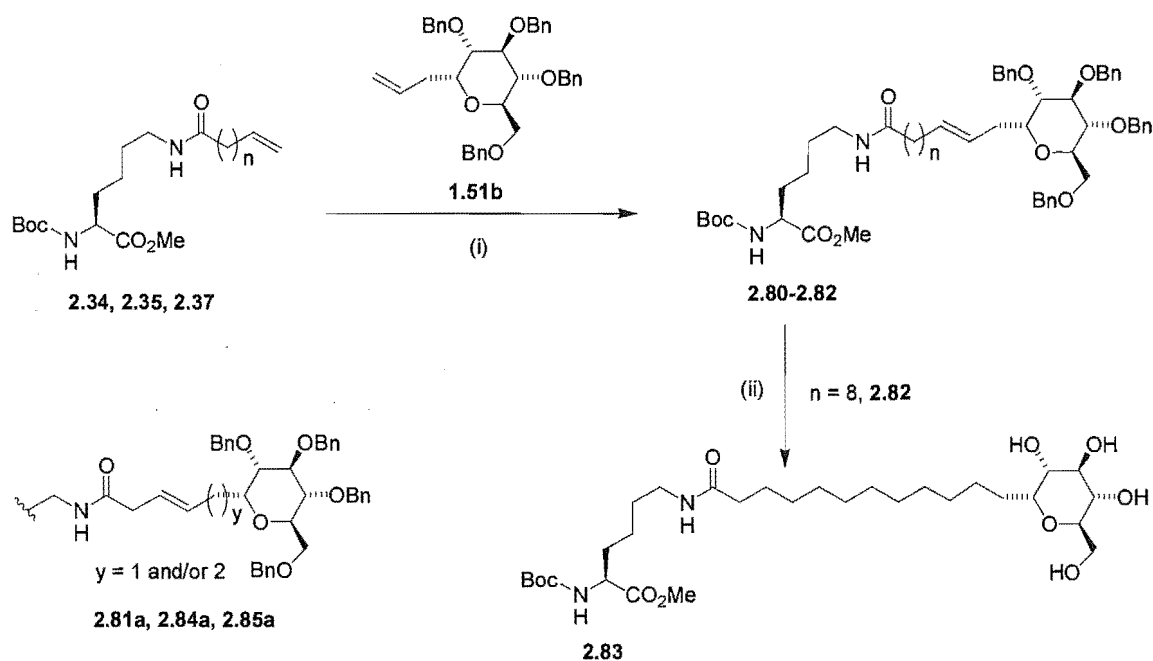
trifluoroetherate gave α -anomer vinyl carbohydrate derivative **1.51b**. The reaction proceeds via nucleophilic attack of the oxonium ion from the preferred axial face.^{43,44}

2.4.2 Cross metathesis of amino acids to carbohydrate compound

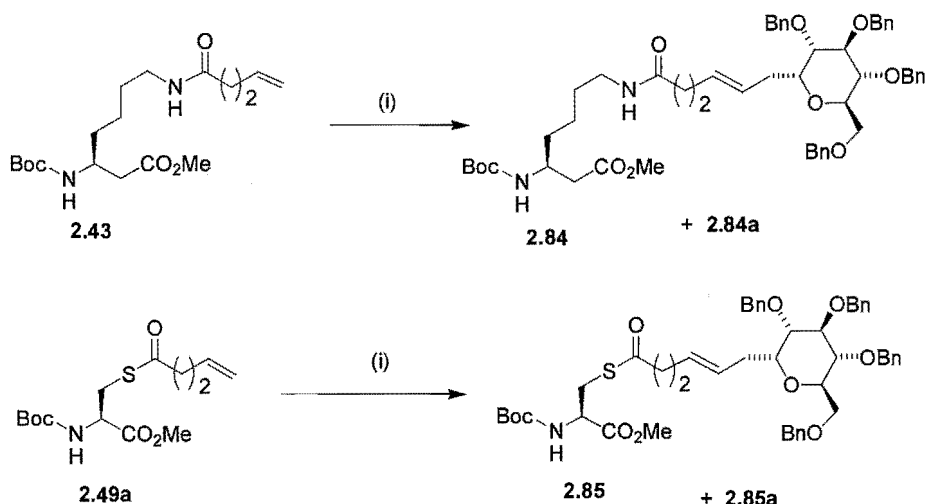
Protected α -glycopyranose carbohydrate **1.51b** was coupled to the three modified lysine amino acids **2.37**, **2.35**, and **2.34**, each of which contains a different acyl chain length (Scheme 2.18). All reactions shown in Scheme 2.18 were carried out with three equivalents of carbohydrate **1.51b** relative to one equivalent of the amino acid-based coupling partner. Consequently the carbohydrate homodimer of **1.51b** was isolated in all reactions. All CM products isolated (Scheme 2.18) were assigned as the *E* configuration, on the basis of the vicinal coupling constant across the double bond (for **2.80**, **2.82**, **2.84**) or on the assumption that *E* is more thermodynamically stable than *Z* (for **2.81**, **2.75**). CM products contained traces of inseparable isomers that were only assigned when minor resonances in the ¹H NMR spectra of CM products could be assigned to a specific type of trace isomer structure.* This was possible for isomers containing an α,γ -unsaturated amide, the structures of which (**2.81a**, **2.84a**, **2.85a**) are shown in Scheme 2.18.

α,β -Unsaturated amide **2.37** was reacted with carbohydrate **1.51b** to give cross coupled product **2.80** in 55% yield. *N*-Pentenoyl **2.35** was coupled to **1.51b** to give an inseparable mixture of **2.81** and **2.81a** (6:1 **2.81**/**2.81a**) in 43% yield. Longer chain *N*-decenoyl lysine **2.34** was also conjugated to **1.51b** to afford **2.82** in a higher yield of 65%. Compound **2.82** can also be regarded as a glycolipoamino acid, as contains both a carbohydrate moiety and a long alkyl chain. Glycoamino acid **2.82** was treated with palladium on carbon under an atmosphere of 74 psi hydrogen gas to give carbohydrate-deprotected and side chain reduced analogue **2.83**. Glycoamino acid **2.83** provides an example of an unprotected carbohydrate linked to the side chain of lysine through a C₁₂ chain.

* Refer to section 2.3.3 for an explanation of isomers that can form during CM due to double bond migration and ring contraction.



n	Amino Acid	CM Product	CM yield
0	2.37	2.80	55%
2	2.35	2.81[#]	43%
8	2.34	2.82	65%



Scheme 2.18. *Reagents and conditions:* (i) Grubbs second generation catalyst **1.17** (20 mol%), **1.51b** (3 equiv), DCM, argon flow, reflux, 6 h, then DMSO (50 equiv rel. to **1.17**), rt, 12 h (**2.84/2.84a** 3:1, 35%), (**2.85/2.85a**, 4:1, 43%). (ii) 10% Pd/C, H₂ (74 psi), EtOH, 24 h (quantitative). [#] Isolated as a mixture of **2.81/2.81a** 6:1.

N-Decenoyl lysine **2.34** was reacted with carbohydrate **1.51b** to give CM product **2.82** in a higher yield (65%) compared to when lysine substrates **2.35** (43%) or **2.37** (55%) were coupled to **1.51b**. This result differs to the terminal alkene model study (Section 2.3), where α,β -unsaturated amide **2.37** gave CM products in the highest yields, likely due to a selective CM reaction between Type II olefin **2.37** (as defined in Table 1.1, Chapter 1) and Type I olefins **2.61** or **2.65**. The observation that CM between carbohydrate **1.51b** and **2.34** gave a higher CM yield than between **1.51b** and **2.37** (Type II olefin) suggested that steric factors were more influential than selectivity issues in this instance. This may be due to the shorter olefin tether length of carbohydrate **1.51b** compared to the longer olefin tethers of **2.61** and **2.65**. The trend of higher CM yields for substrates containing longer olefin tether lengths are used has previously been reported, for example see Schemes 1.1 and 1.13, Chapter 1.

*N*_E-Pentenoyl β -lysine **2.43** was coupled to carbohydrate **1.51b**, to give a mixture of glycoamino acids **2.84** and **2.84a** (3:1 ratio respectively) in 35% yield (Scheme 2.18). This demonstrated the applicability of CM methods to β -amino acids, which are important variations of natural α -amino acids. Modified *S*-pentenoyl cysteine **2.49a** was also reacted with **1.51b** in a CM reaction to afford coupled products **2.85** and **2.85a** (4:1 ratio respectively) in 43% yield, the same yield as was observed in the CM reaction of **2.35** with **1.51b**. Carbohydrate **1.51b** was reacted with *O*-decenoyl serine **2.46** under CM coupling conditions to give a crude reaction mixture that contained evidence of only coupled RHC=CHR resonances in the ¹H NMR spectrum, indicating an efficient CM coupling had occurred. However the cross-coupled product could not be purified from the carbohydrate homodimer using silica gel-based column chromatography.

The ¹H, ¹³C, and/or HRMS spectra of CM products in Scheme 2.18 showed evidence of trace isomers as an inseparable mixture following chromatography. These isomers were only assigned if a distinguishable doublet at approximately δ_H 2.9 ppm corresponding to an α,γ -unsaturated amide could be observed in the ¹H NMR spectra of CM products, for example in the cases of **2.81a**, **2.84a**, and **2.85a**. These isomers were assigned on the basis that they contained an α,γ -unsaturated amide functional group as a result of double bond migration, but it was not determined if the observed isomer also corresponded to a chain contracted product. However small peaks corresponding to a compound of one less

methylene unit compared to the major CM product were observed in each of the HRMS of products shown in Scheme 2.15, apart from 2.80. This indicated that traces of chain contracted compounds were present along with the major CM product.

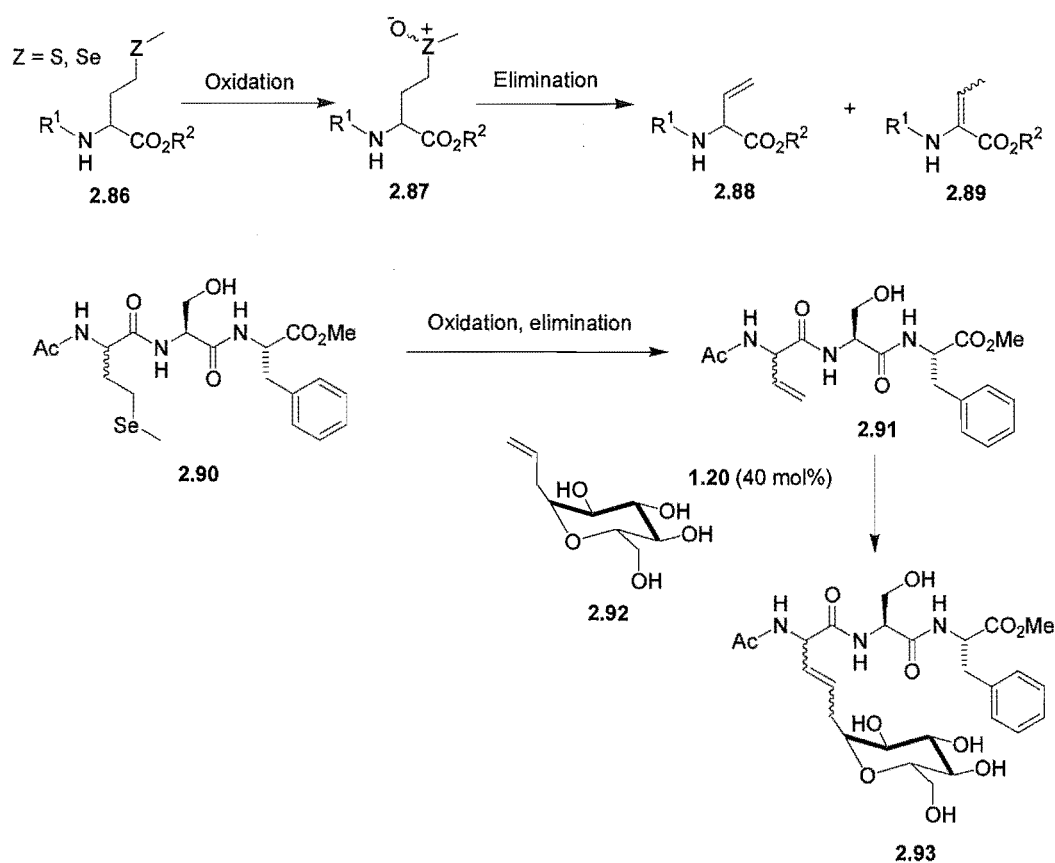
CM products shown in Scheme 2.18 represent examples of glycoamino acids in which the amino acid side-chain is linked to the anomeric position of a carbohydrate by a carbon chain. This project is the first example of an acyl tether containing an olefin attached to the side-chain of an amino acid being linked via CM to a carbohydrate to give a glycoamino acid. This methodology has some advantages over previously reported methods discussed in Sections 2.1 and 1.5.1. The synthesis of α -carbon substituted amino acids such as allylglycine invariably creates a new stereocentre, the chirality of which must be controlled to produce one enantiomer. The acyl side-chain approach used in this study to install the double bond tether does not form a new stereocentre, so the chirality at the natural amino acid α -carbon centre is not an issue.

Another advantage of carrying out CM on an amino acid acylated with an olefin tether as described in this study is the ability to control the tether length. This was demonstrated in Scheme 2.18 where three different acyl tether lengths of 2.37, 2.35, and 2.34 all underwent CM with carbohydrate 1.51b in similar yields. Other types of alkene attachments, for example α -carbon substituted attachments such as in allylglycine, have limited tether lengths and often rely on the defined three carbon conjugated system of allyl bromide to install the double bond tether.

A patent was published by Davis and Kramer in January 2005 describing a general method for the attachment of amino acids or peptides to unprotected carbohydrates using CM.¹⁷ Amino acids substituted with an olefin tether at the α -carbon position are preferably used in this patent, for example commercially available vinyl glycine. In particular this patent focuses on the oxidation of a methionine or selenomethionine side chain 2.86 to the corresponding sulfoxide or selenoxide 2.87, followed by elimination at an elevated temperature/pressure to give vinyl amino acid 2.88 (Scheme 2.19). The elimination reaction also produced approximately the same amount of α,β -dehydro side product 2.89. The modification of methionine or selenomethionine to contain a double bond can also be

carried out in pre-assembled peptides, for example peptide **2.90** can be modified in two steps by oxidation and elimination to give **2.91**.

The modified vinyl amino acids or peptides (e.g. **2.91**) were coupled to unprotected carbohydrates such as **2.92** by treatment with second generation recyclable catalyst **1.20** in methanol with microwave heating to give glycopeptide products (e.g. **2.93**). The CM method shown in Scheme 2.19 does not overlap with the work carried out in this thesis, as only unprotected carbohydrates are covered under the Davis *et al* patent.¹⁷



Scheme 2.19.

There are drawbacks to the method shown in Scheme 2.19. Firstly the preparation of **2.88** also gives α,β-dehydro **2.89** as a by-product that will not react under CM conditions. Secondly a peptide must contain a methionine or selenomethionine to enable the introduction of the double bond functionality. The authors use peptides and proteins that contain one or more methionine residues, or alternatively a methionine or selenomethionine residue was installed via site-directed mutagenesis or a SetMet culture

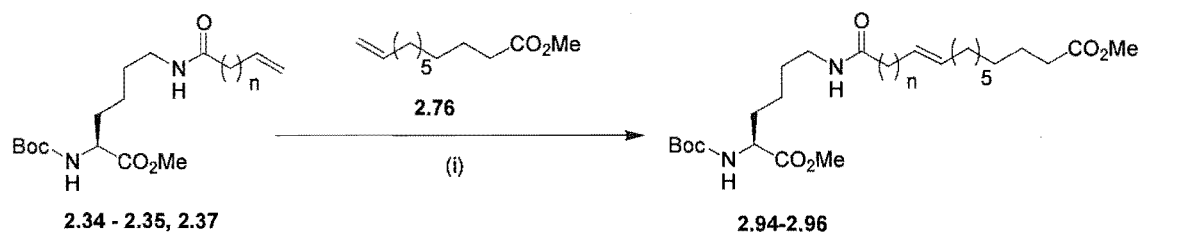
medium during peptide synthesis. These extra steps add complexity, compared to the methodology used in this thesis for alkene attachment that required only one mild and reliable reaction to install the double bond moiety onto the side chain of natural amino acids more commonly found in many peptides. Thirdly the elimination reaction to give **2.88** or **2.91** shown in Scheme **2.19** requires high temperature/pressure conditions that could degrade some peptides.

The method shown in Scheme **2.19** is not as versatile as the methodology described in this thesis since a methionine or selenomethionine residue is required, and the olefin tether length that is substituted at the amino acid α -carbon can only be a vinyl tether of non-variable length. In this thesis, different tether lengths in glycoamino acids between the amino acid and carbohydrate component can be achieved, simply by side chain acylation using different lengths of alkene acids. The method described in this thesis also allows for olefin tether lengths to be installed into many types of natural amino acids, for example lysine, serine, and cysteine.

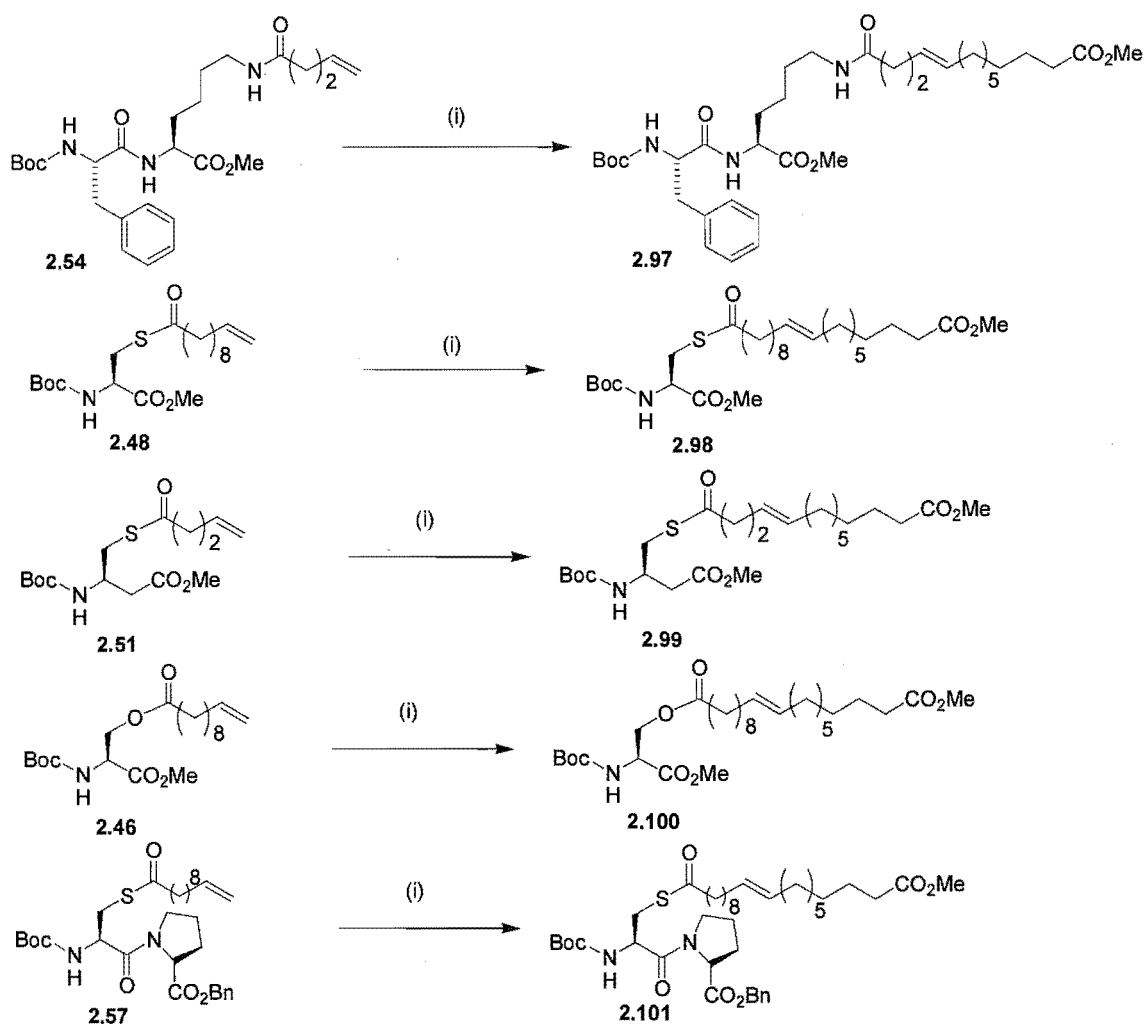
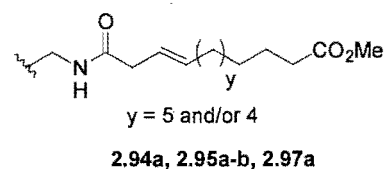
2.4.3 Cross metathesis of amino acids to fatty acid compound

Three lysine-based amino acids **2.37**, **2.35**, and **2.34**, each containing a different olefin tether length, were coupled to protected fatty acid **2.76** using CM (Scheme 2.20). Fatty acid **2.76** was used in three fold excess and the corresponding homodimer of **2.76** was also isolated. α,β -Unsaturated amide **2.37** was reacted with fatty acid **2.76** to give lipoamino acid **2.94** in 68% yield. CM product **2.94** also contained trace isomers (**2.94/2.94a** 12:1), presumably due to double bond migration of CM product **2.94**. *N*-Pentenoyl lysine **2.35** was also coupled to **2.76** to give an inseparable mixture of **2.95** and traces of double bond migrated products **2.95a–b** (34:4:1 **2.95/2.95a/2.95b**) in 48% yield. *N*-Decenoyl **2.34**, containing a longer olefin tether, was coupled to **2.76** to afford **2.96** in 64% yield. CM product **2.94**, which contains an α,β -unsaturated amide, was formed in higher yield compared to both **2.95** and **2.96**. The same trend was observed in the terminal alkene model study (Section 2.3), thought to be due to a selective CM reaction between two olefins of different Types, as defined in Table 1.1, Chapter 1. *N*-Decenoyl **2.96** was formed in slightly lower yield than **2.94** but still higher than for **2.95**, presumably due to steric reasons.

N-Pentenoyl dipeptide **2.54** was conjugated to fatty acid **2.76** to afford a mixture of **2.97** and **2.97a** (4:1 **2.97/2.97a**) in 41% yield, a result comparable to the 48% yield obtained for single amino acid *N*-pentenoyl **2.95** (Scheme 2.20). This demonstrates the applicability of this methodology to larger molecular architectures. CM of *S*-decenoyl cysteine **2.48** to **2.76** was carried out to give lipoamino acid **2.98** in 61% yield, a result comparable result to the 64% yield for *N*-decenoyl lysine product **2.96**. β -Cysteine derivative **2.51** was successfully coupled to fatty acid **2.76** to afford **2.99** in 59% yield. Conjugate **2.99** is an example of a β -lipoamino acid (a β -amino acid linked to a fatty acid), the basis of which could be a useful synthetic conjugate in biological systems as β -amino acids are hydrolytically more stable than α -amino acid analogues,⁴⁵ and are becoming important peptidomimetic structures for therapeutic applications. *S*-Decenoyl cys-pro dipeptide **2.57** was coupled to **2.76** via CM to give lipopeptide **2.101** in 43% yield. *O*-Decenoyl serine **2.46** was also reacted with **2.76** to afford CM product **2.100** in 73% yield, the highest yield for all reactions shown in Scheme 2.20.



n	Amino Acid	CM Product	CM yield
0	2.37	2.94	68%
2	2.35	2.95[#]	48%
8	2.34	2.96	64%



Scheme 2.20. Reagents and conditions: (i) Catalyst **1.17** (20 mol%), **2.76** (3 equiv), DCM, argon flow, reflux, 6 h, then DMSO (50 equiv rel. to **1.17**), rt, 12 h (**2.97/2.97a** 4:1, 41%), (**2.98**, 61%), (**2.99**, 59%), (**2.100**, 73%), (**2.101**, 43%). [#] Isolated as a mixture of **2.95/2.95a/2.95b** 34:4:1.

The reaction of acetyl dipeptide **2.59** with **2.76** did not give rise to the CM product, presumably because the dipeptide was only sparingly soluble in DCM. Further reactions with **2.59** were not attempted as available catalyst **1.17** was only soluble in solvents such as DCM.

CM products shown in Scheme **2.20** were all assigned as *E*. This was determined on the basis of the vicinal coupling constant across the double bond for **2.94**, **2.95**, **2.97** and **2.99**, or was assumed for thermodynamic reasons. Small resonances due to trace isomers were observed in the ^1H NMR spectra of **2.94** – **2.101**. The only isomers that could be assigned were **2.94a**, **2.95a-b**, and **2.97a**, which contain an α,γ -unsaturated amide as a result of double bond migration. Analysis of CM products **2.95-2.101** by mass spectrometry revealed small peaks that corresponded to species with one less methylene unit compared to the major CM products, consistent with alkyl chain contracted products.

The lipoamino acid and lipopeptides shown in Scheme **2.20** are all novel structures. This is the first report of the use of side-chain acylation followed by CM to attach lysine, serine, and cysteine to a fatty acid. The only other example of attachment of a fatty acid to an amino acid side-chain via CM is to allyl-serine compounds, for example **1.65**, Scheme **1.10**. Previous literature methods for lipoamino acid and lipopeptide synthesis include attachment of a fatty acid chain to the amino acid α -carbon (e.g. see Scheme **2.5**).²¹ Advantages of the methodology developed in this thesis include the attachment of a fatty acid without creating a stereocentre, and the ability to install the fatty acid group either pre- or post-peptide synthesis. Installing a fatty acid onto an olefinic side chain of an amino acid after peptide synthesis could be an advantage, for example to prevent the fatty acid group interfering with standard SPPS techniques.

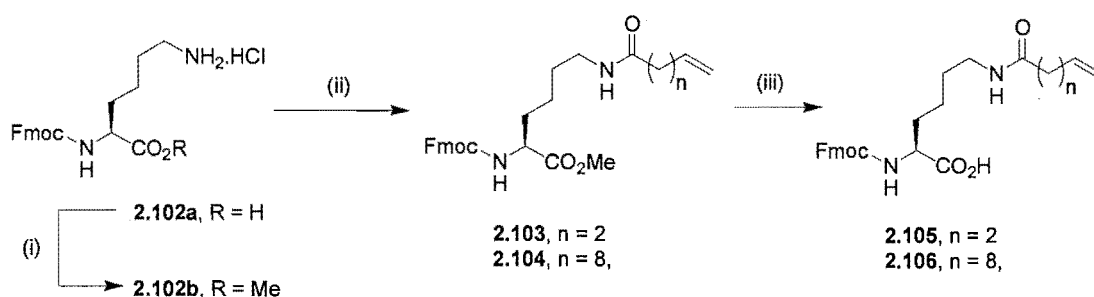
In summary, a new and important method that allows conjugation of a suitably functionalised fatty acid or carbohydrate to the acylated side chain of the natural amino acids lysine, cysteine, or serine has been identified. This methodology should be amenable to a wide range of other biologically important olefins. The ability to couple these modified amino acids to carbohydrates is particularly significant since it paves the way for the preparation of important peptide-carbohydrate complexes.

2.5 APPLICATION OF CROSS METATHESIS TO SOLID-PHASE CHEMSITRY

2.5.1 Attempted solid-phase cross metathesis

CM of modified amino acids to terminal alkene **2.65** on solid phase was attempted as an alternative to solution phase reactions. This offers the advantage of easier and more thorough purification, as CM products attached to the resin could be vigorously washed before cleavage and isolation. The Fmoc group was selected for protection of the backbone amine for use in solid-phase peptide synthesis (SPPS),⁴⁶ rather than a Boc group as was used in solution-phase CM.

Fmoc-protected **2.105** and **2.106** were prepared as substrates for solid-phase CM reactions (Scheme 2.21). Commercially available Fmoc-*L*-Lys-COOH **2.102a** was reacted with methanol in the presence of Amberlyst 15 ion exchange resin to give methyl ester **2.102b** in quantitative yield. The side-chain amine of **2.102b** was coupled to 4-pentenoic or 10-decenoic acid by treatment with EDCI and HOBT to give *N*-pentenoyl **2.103** or *N*-decenoyl **2.104** respectively. The methyl esters of **2.103** and **2.104** were hydrolysed with aqueous lithium hydroxide to give the desired N_α -Fmoc-protected carboxylic acids **2.105** and **2.106**, which possess the required side-chain olefin tether.



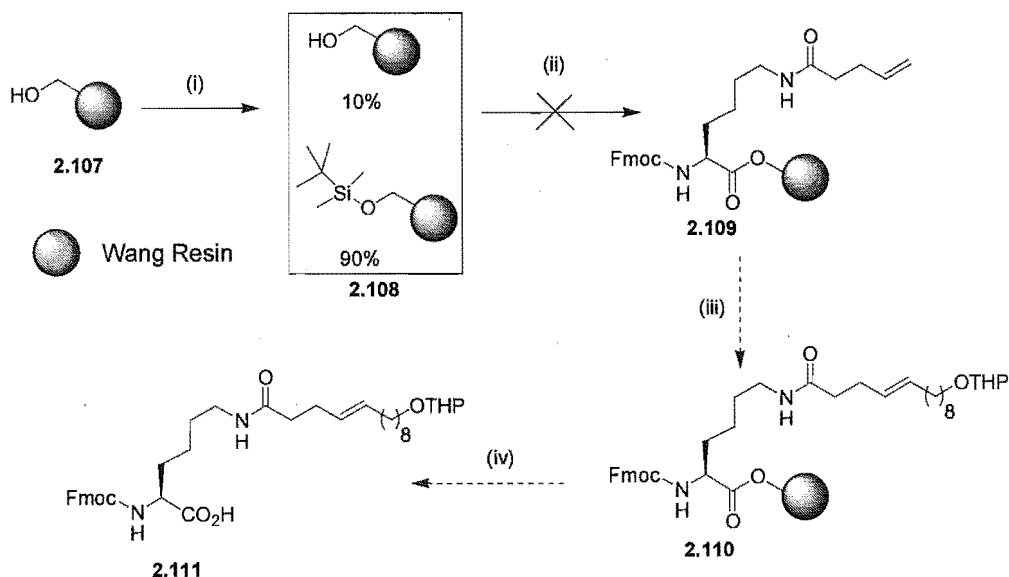
Scheme 2.21. Reagents and Conditions: (i) Amberlyst 15 ion exchange resin, MeOH, rt, 24 h (99%). (ii) EDCI, HOBT, DIPEA, DCM, rt, 16 h, 4-pentenoic acid (**2.103**, 66%), or 10-decenoic acid (**2.104**, 60%). (iii) LiOH_(aq), THF, 0°C, 40 min (**2.106**, 96%).

N_α -Fmoc- N_ϵ -pentenoyl-Lys-OH **2.105** was unfortunately completely insoluble in petroleum ether, ethyl acetate, DCM, THF, DMF, water, and only sparingly soluble in

methanol, all at both rt and reflux. Carboxylic acid derivative **2.105** was not characterised due to insolubility, but was presumed to be the structure present following treatment of **2.103** with lithium hydroxide. A solvent often used in SPPS is *N*-methylpyrrolidone (NMP) - even this did not solubilise **2.105**. Therefore *N*-pentenoyl lysine **2.105** was not suitable for solid-phase chemistry due to its insolubility. Longer chain *N*-decenoyl carboxylic acid **2.106** was sparingly soluble in DCM and DMF so solid-phase chemistry with this derivative was attempted.

Solid phase metathesis reactions can be carried out using either resin-bound substrates or a resin-bound catalyst. A CM method that entailed reaction of a resin-bound olefin substrate with a solution containing the other olefin substrate and Grubbs second generation catalyst **1.17** was selected for this project. Solid-phase CM carried out in this way requires a low loaded concentration of the resin-bound olefin to avoid the competing homodimerisation (or pseudo-RCM) reaction. This was achieved by capping a large portion of the resin binding sites before loading the CM olefin substrate onto the remaining sites.

The solid-phase metathesis procedures used were based upon work carried out by Gibson and co-workers.³⁴ Wang resin **2.107** was treated with 0.9 equivalents of tertiary butyl dimethyl silyl chloride to give resin **2.108**, that was presumed to contain 90% capped and 10% free alcohol binding sites (Scheme 2.22). Resin **2.108** was reacted with Fmoc-*L*-Lys-OH **2.106**, EDCI, and DMAP in an effort to give resin-bound lysine derivative **2.109**, which was treated with a solution of terminal alkene **2.65** and catalyst **1.17** in an effort to produce the resin-bound CM product **2.110**. Resin **2.110** was then thoroughly washed, treated with TFA, and the filtrate and washings collected, however CM product **2.111** was not isolated. The entire sequence of reactions shown in Scheme 2.22 was repeated twice more to give the same end result. The ¹H NMR spectrum of the filtrate following TFA treatment of resin **2.110** was complex, and resonances corresponding to either **2.111** or starting material **2.106** were not observed. This was not helped by the fact the reaction sequence shown in Scheme 2.22 was carried out on a small scale relative to the amounts of olefin substrates used as resin **2.108** had only 10% of available binding sites. The scale-up of the reaction was limited due to equipment restrictions and the amount of Wang resin available.

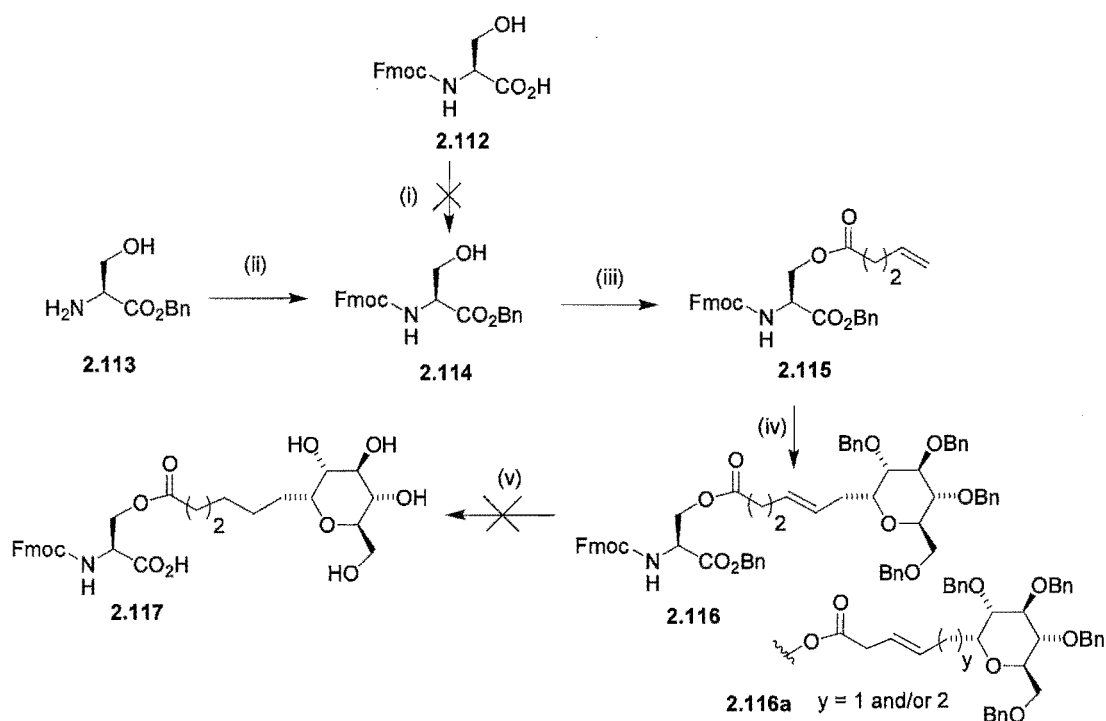


Scheme 2.22. *Reagents and Conditions:* (i) 0.9 eq $t\text{BuMe}_2\text{SiCl}$, Et_3N , DCM, shake, rt, 24 h. (ii) **2.106**, EDCI, DMAP, DCM, shake, rt, 24 h. (iii) Catalyst **1.17**, **2.65**, reflux, 24 h. (iv) TFA, DCM. Wang resin **2.107** (Novabiochem, 200-400 mesh, loading 0.60 mmole/g resin).

Studies were next carried out to determine whether or not **2.106** was binding to the Wang resin, as **2.106** was only sparingly soluble in DCM. The coupling of **2.106** directly to an uncapped sample of Wang resin **2.107** was attempted following standard SPPS protocol.⁴⁶ N_α -Fmoc- N_ϵ -decanoyl-lysine **2.106** was only sparingly soluble in various combinations of DCM, DMF, and NMP however it was hoped that solubility in 1:1 DMF/DCM was sufficient to enable some binding of **2.106** to resin **2.107**. After treatment of **2.107** with **2.106**, EDCI, and DMAP the resin was treated with TFA, but ^1H NMR spectra (CDCl_3) of the resulting filtrate did not contain any type of lysine-based compound, indicating that **2.106** was not binding on to resin **2.107**. Therefore it was assumed that CM product **2.111** was not isolated for this reason.

2.5.2 Synthesis of solid phase peptide synthesis building blocks

The development of a glycoamino acid building block for use in SPPS of glycopeptides was another aim of this project. Attempts at solid-phase CM discussed in section 2.5.1 suggested that Fmoc protected lysine-based amino acids such as **2.105** and **2.106** were not suitable for SPPS application due to insolubility. Instead the more non-polar serine-based modified amino acid **2.115** was prepared, again with the Fmoc amine protecting group that is commonly used in SPPS. The synthesis of Fmoc-*L*-Ser-OBn **2.114** was initially attempted by reaction of commercially available Fmoc-*L*-Ser-OH **2.112** with benzyl alcohol in the presence of *p*-toluene sulfonic acid monohydrate (Scheme 2.23), but this gave complex mixtures of inseparable products. Instead commercially available *L*-Ser-OBn **2.113** was reacted with Fmoc-chloride to give **2.114** in 90% yield.



Scheme 2.23. Reagents and Conditions: (i) Benzyl alcohol, *p*-toluene sulfonic acid monohydrate, CHCl₃, reflux, 24 h. (ii) Fmoc-Cl, DIPEA, DCM, 1 h (90%). (iii) EDCl, HOBt, DIPEA, DCM, 18 h (94%). (iv) Catalyst **1.17** (20 mol%), **1.51b** (3 equiv), DCM, nitrogen flow, reflux 6 h, then DMSO (50 equiv rel. to **1.17**), rt, 12 h (36%). (v) 10% Pd/C, H₂, MeOH/ethyl acetate.

The side-chain alcohol of **2.114** was acylated using 4-pentenoic with EDCI and HOBt to give **2.115** in 94% yield. The CM substrate **2.115** was then reacted with **1.51b** in the presence of catalyst **1.17** to give an inseparable 12:1 mixture of **2.116** and **2.116a** respectively in 36% yield. The preparation of glycoamino acid **2.116** demonstrates that the CM methods used in this thesis are amenable to the Fmoc protecting group, which is an important and useful functional group for use in SPPS. Compound **2.116** was submitted to hydrogenation conditions using palladium on carbon under an atmosphere of hydrogen in an effort to produce the benzyl deprotected and double bond reduced product **2.117**, but this gave a complex mixture of products that could not be elucidated. It is possible that the hydrogenation conditions also cleaved the *N*-Fmoc protecting group, as has been reported in some instances.⁴⁷ Therefore the synthesis of an *N*_α-Fmoc protected carboxylic acid SPPS building block requires the use of a different *C*-terminal protecting group that can be cleaved under conditions where the *N*-Fmoc group is stable. Due to time constraints the synthesis of other glycoamino acid SPPS building blocks was not attempted.

2.6 CONCLUSION AND FUTURE WORK

The side chains of lysine, serine, and cysteine amino acids were acylated with either 10-decenoic acid, 4-pentenoic acid, vinyl acetic acid, or acryloyl chloride to give substrates suitable for CM elaboration. These included those based on lysine (**2.34** – **2.37**), β -lysine (**2.43**), serine (**2.45**, **2.46**), cysteine (**2.48**, **2.49a**), β -cysteine (**2.51**), as well as dipeptides based on Phe-Lys (**2.54**) and Cys-Pro (**2.57**) scaffolds. This approach is versatile in that a range of olefin tether lengths from C₃ to C₁₁ can be controllably introduced using a mild acylation reaction. A model study of the CM between amino acid-based substrates (**2.34** – **2.37**, **2.48**, **2.54**) and terminal alkenes (**2.61** and **2.65**) was carried out, to give CM products (**2.66** – **2.74**) in yields of 36% – 91%. This demonstrated that CM between side-chain acylated amino acids with a controllable olefin tether length and target compounds was a viable and versatile general method.

Side chain acylated amino acids **2.34** – **2.37**, **2.43**, **2.49a** that contain a suitable olefin tether were then each coupled to carbohydrate **1.51b** using catalyst **1.17** to give glycoamino acids **2.80** – **2.85** as the CM products. Amino acid-based substrates **2.34** – **2.37**, **2.54**, **2.48**, **2.51**, **2.46** and **2.57** were coupled to fatty acid derivative **2.76** using catalyst **1.17** to give lipoamino acids **2.94** – **2.101** as the CM products. The preparation of these glyco- and lipoamino acids again demonstrated the versatility of this general methodology, in that amino acids of different types and with variable but controllable olefin tether lengths can be coupled via CM to suitably modified target biological molecules. Synthesis of larger molecular architectures such as Phe-Lys lipopeptide **2.97** in a similar yield to single amino acid lysine lipoamino acid **2.95** demonstrated that this method is applicable to larger molecular architectures. Solid phase CM was attempted between *N*_α-Fmoc-*N*_ε-decenoyl-lysine substrate **2.106** and terminal alkene **2.65**, but no coupled product could be isolated due the insolubility of **2.106** in DCM/DMF.

Future work in this area could involve applying the general methodology developed for amino acid- and dipeptide-based CM substrates to larger peptides and eventually proteins. This could be achieved using reaction conditions such as catalyst **1.20** in methanol. In principle any amino acid, peptide, or protein with a side-chain that can be derivatised to contain an olefin could be linked using CM to any target molecule (e.g. a hormone or complex natural product) that is also suitably modified to contain an olefin.

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CHAPTER THREE

CROSS METATHESIS DIMERS

3.1 INTRODUCTION

Cross linking in peptides is widespread and has many important implications. It can result in structural changes that impart modified biological function, for example in blood clotting and collagen. Nature has evolved a number of general methods for the formation of these linkages, for example the cross-linking of lysine residues in the Maillard reaction,¹ and the formation of disulfide bonds in the case of cysteine. Synthetic mimics of natural protein cross-links are of interest for many reasons, including the probing of biological structure/function relationships or the development of therapeutics. Section 3.2 describes the preparation of a novel series of homo- and hetero-amino acid dimers via CM coupling. These dimers were synthesised to expand the scope of the general CM coupling strategy described in Chapter 2, which is a versatile method involving side-chain acylation with varying carboxylic acids to install the olefin tether. The following section outlines the synthesis of selected amino acid and peptide cross-linked dimers and the coupling reactions that have been used for dimerisation.

Dityrosines such as 3.2 are naturally occurring biaryl cross-links that result from the ortho coupling of two tyrosyl radicals, and are found in proteins such as RNase A enzyme and in the eye lens α - and γ B-crystallins.² Synthetic cross linking of tyrosine residues is also of interest as tyrosine-like dimers are often found as part of natural product scaffolds that have potent biological activities.³

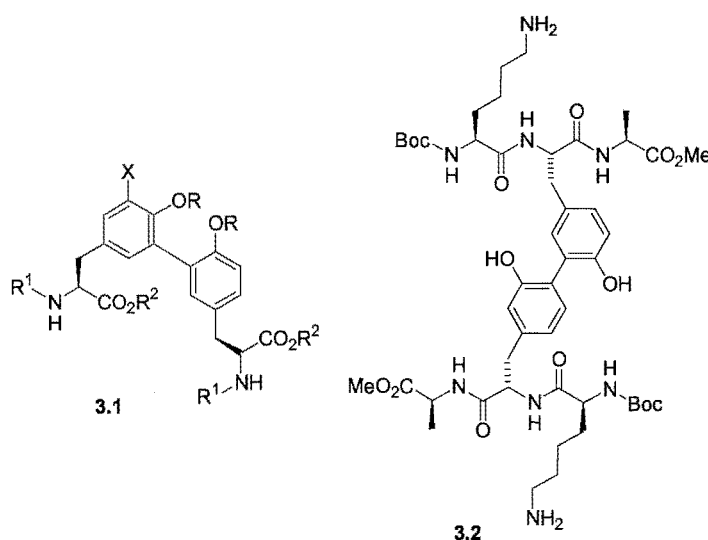
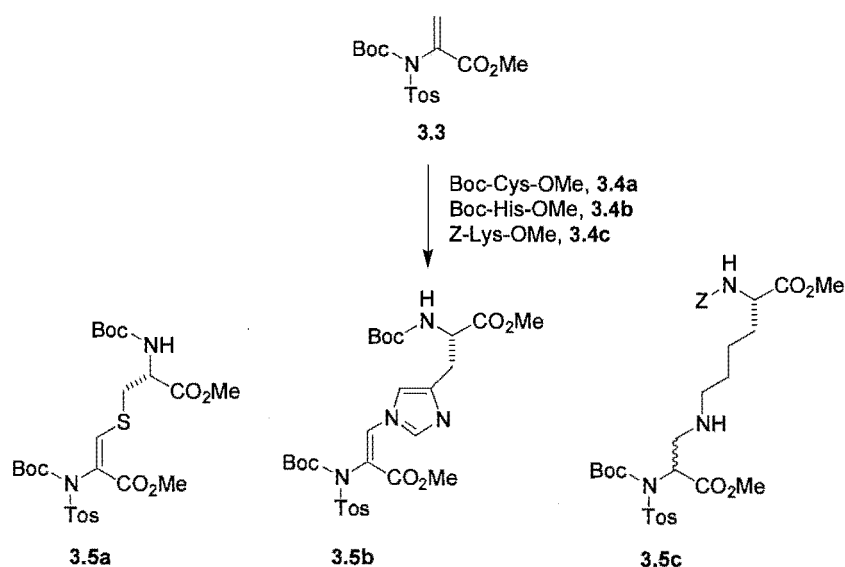


Figure 3.1.

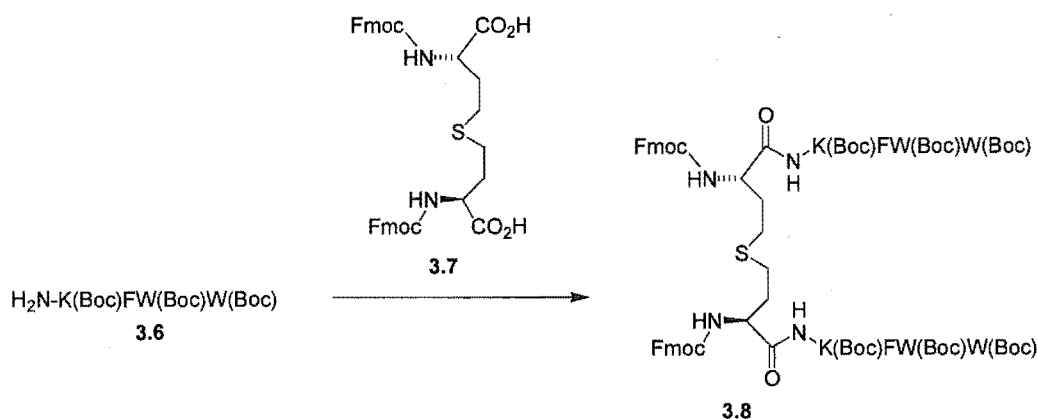
Carbon-carbon cross linked dityrosine compounds such as **3.1** have been synthesised using Stille coupling conditions (Figure 3.1).⁴ Dityrosine cross-linked peptide **3.2** was synthesized using a Miyaura-Suzuki coupling reaction.⁵ Tyrosine dimers have also been synthesised using a phenol oxidation approach.⁶

Side-chain cross-linked amino acids have been synthesised by nucleophilic attack of thiol (**3.4a**), imidazole (**3.4b**) or amine (**3.4c**) side chain functional groups to dehydroalanine **3.3** to give **3.5a-c** respectively (Scheme 3.1).⁷

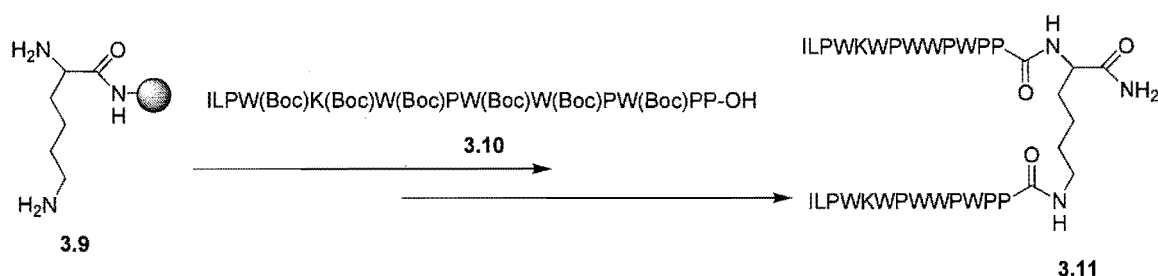


Scheme 3.1.

Ligand-induced receptor and protein dimerisation or oligomerization has been identified as a general mechanism for signal transduction in proteins such as tyrosine kinase, serine/threonine kinase, and class I cytokine receptors.⁸ Compounds that mimic this natural dimer structure have been synthesized as effective enzyme inhibitors, for example HIV-1 integrase inhibitor **3.8** (Scheme 3.2).⁹ Hexapeptide dimer **3.8** was constructed by linking peptide **3.6** with cysteine dimer **3.7**, synthesized itself from Boc₂-L-homocysteine by a sulfur extrusion reaction. The inhibitor **3.8** is more potent than monomeric **3.6**, presumably because the bivalent inhibitor simultaneously occupies two neighboring catalytic sites in the HIV-1 integrase oligomer.⁹ Dimeric HIV-1 integrase inhibitors have also been linked using a lysine residue, for example resin-bound **3.9** was coupled to HIV-1 integrase peptide inhibitor **3.10** to give dimeric **3.11**, which showed higher biological activity compared to the monomer (Scheme 3.3).¹⁰

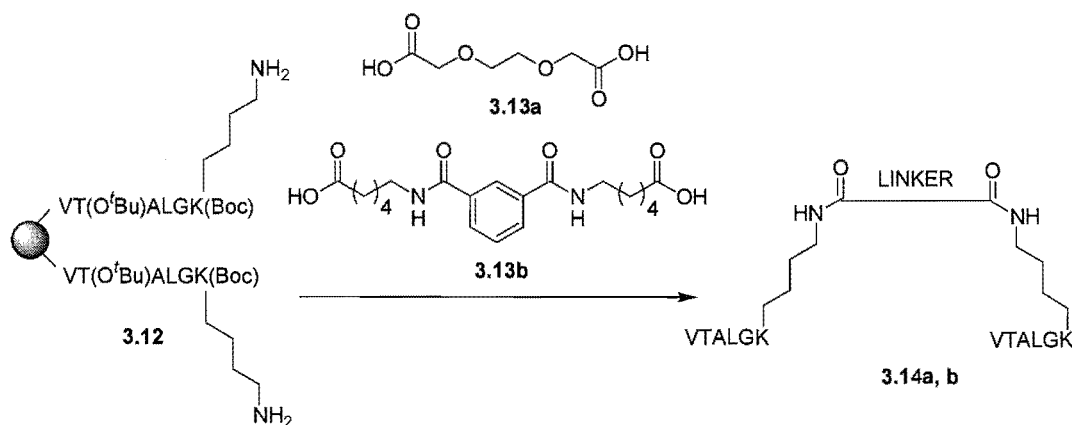


Scheme 3.2.



Scheme 3.3.

Peptides have been dimerized by linking the side chains of terminal lysine residues. For example resin bound peptide **3.12** was dimerized using different types of linkers (**3.13a-b**) to give dimers **3.14a-b** (Scheme 3.4).¹¹ Side chain dimerisation leaves both *N*- and *C*-termini unsubstituted, which can be advantageous as these groups can be involved in important interactions with biological targets.¹²



Scheme 3.4.

3.1.1 Formation of amino acid and peptide dimers using cross metathesis

The first reported example of an amino acid dimer synthesised using CM was in 1997, where dimers **1.34b-c** were reported as side product homodimers in the CM of two different alkenes (Chapter One, Scheme 1.1).¹³ There are also examples where the CM dimer is the intended product, for example in the synthesis of **1.63a**, **1.77**, **1.72** and **1.67** (Figure 3.2) that were discussed in Chapter One (Schemes 1.10, 1.12, 1.14).¹⁴ These symmetrical dimers were cross linked through a serine or tyrosine side-chain *O*-allyl tether. The synthesis of pentapeptide **1.67** demonstrates the use of CM homodimerisation for the construction of highly functionalized, large peptide dimers.

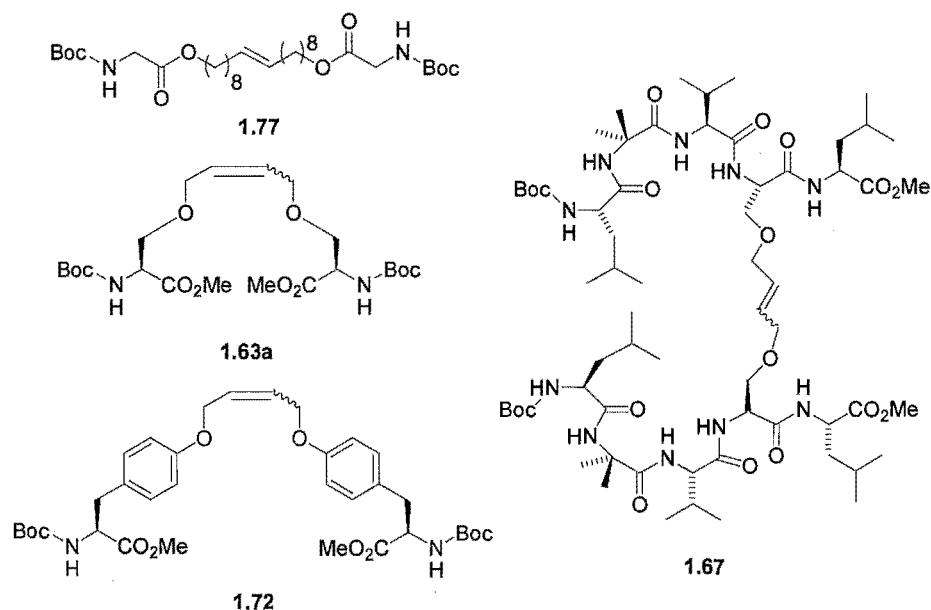
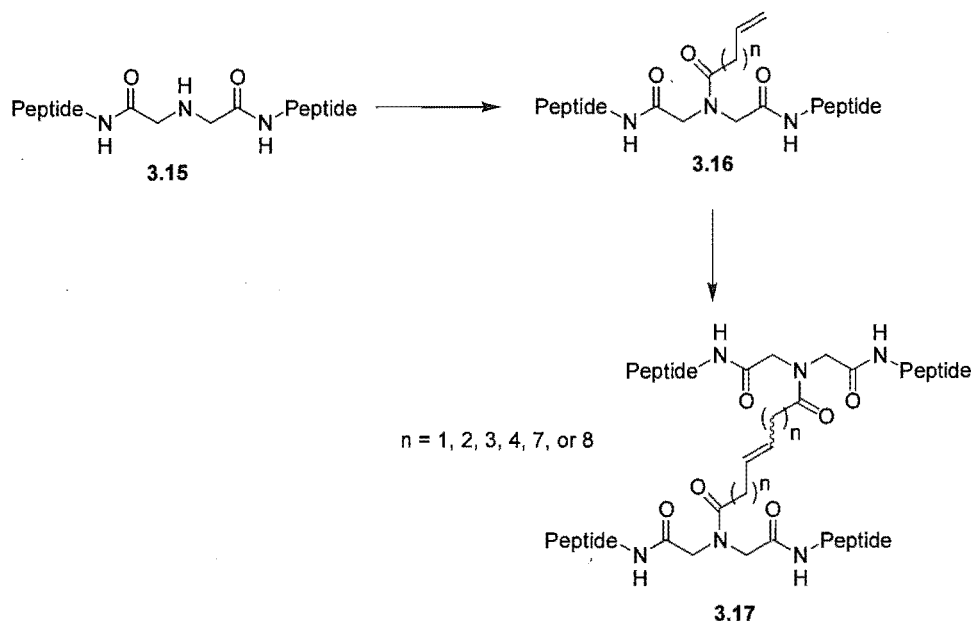


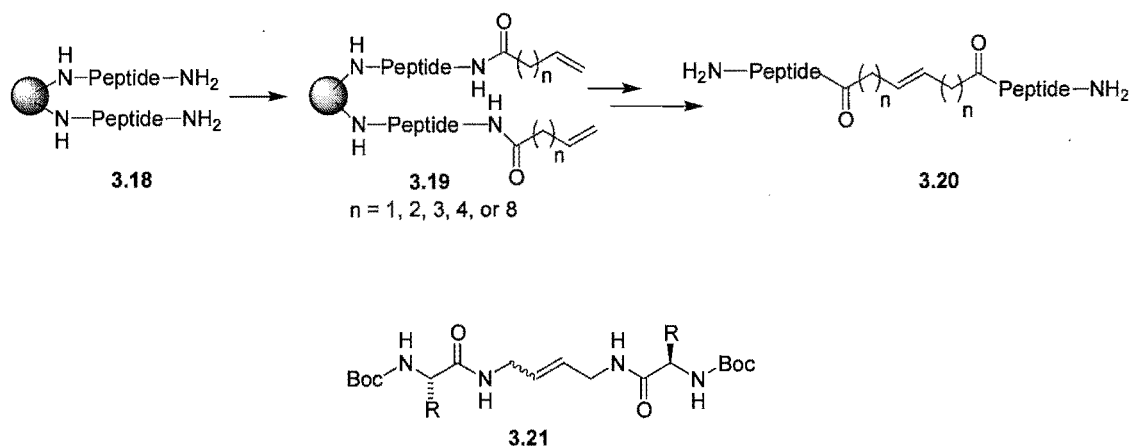
Figure 3.2.

Libraries of peptide dimers have been constructed using solution-phase combinatorial CM.^{15,16} For example peptides **3.15** were coupled to an alkyl carboxylic acid through an *N*-peptide backbone linkage to give **3.16**, which were then dimerized to produce cross-linked dimers **3.17** (Scheme 3.5). Dimeric peptides have also been synthesised using an *N*-terminal linkage. The free amines of resin bound peptides **3.18** were acylated with a range of olefin-containing carboxylic acids to give **3.19**, which were dimerized using CM to give coupled products **3.20** (Scheme 3.6).¹⁷ Similar backbone-tethered compounds such as **3.21**

have also been constructed, in studies directed towards the use of CM for the construction of dynamic combinatorial libraries.¹⁸



Scheme 3.5.



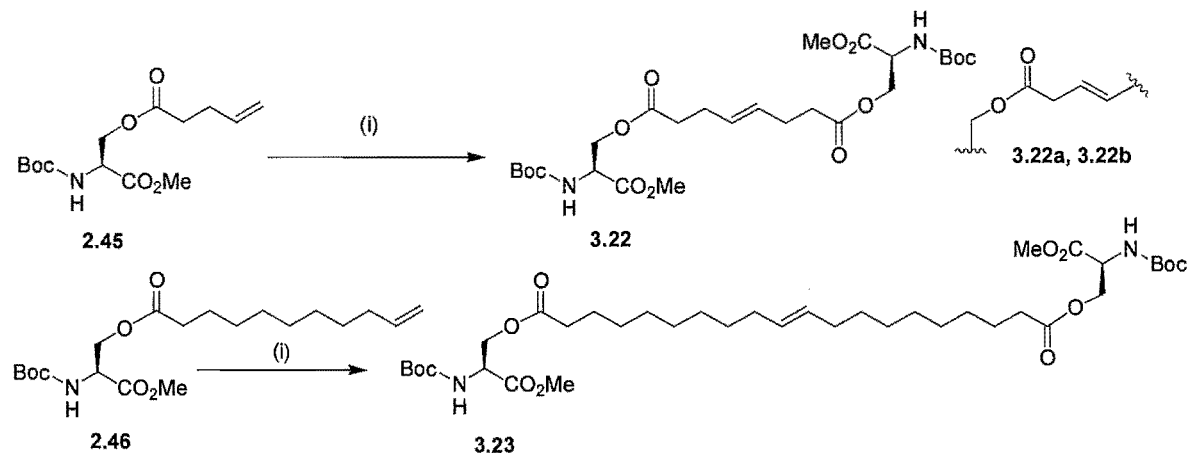
Scheme 3.6.

Chapter 2 demonstrated that CM using amino acid-based substrates that contain an acyl side-chain tether of variable length was a highly versatile and useful procedure. These side-chain acylated amino acid substrates were also utilized for the synthesis of CM homo- and heterodimers, as is outlined in section 3.2, to further extend the scope of this general methodology. Comparisons between the strategy used in this thesis and literature examples discussed in section 3.1 are outlined at the end of section 3.2.

3.2 CROSS METATHESIS DIMERS

3.2.1 Symmetrical Dimers

Symmetrical dimers were constructed by the self-metathesis or homodimerisation CM coupling of amino acid-based substrates (e.g. **2.45**, **2.46**) that contain an acyl side-chain olefin tether. These included two serine symmetrical dimers, each with a different cross-linked tether length (**3.22** and **3.23**, Scheme 3.7). *O*-Pentenoyl serine **2.45**, prepared as in Scheme 2.10, Chapter 2, was treated with Grubbs second generation catalyst **1.17** to give homodimer **3.22** (86% yield) which contained traces of two double bond migrated products (**3.22a** and **b**) (20:4:1 by ^1H NMR) following silica gel-based column chromatography. These trace isomers (**3.22a** and **b**) were assigned by the presence of minor doublets at δ_{H} 3.01 ppm and 3.09 ppm in the ^1H NMR spectrum of the product mixture, that were presumably due to double bond migrated compounds that contain an α,γ -unsaturated amide.* Catalyst **1.17** was used in 20 mol% relative to two equivalents of the amino acid monomer in all CM dimerisation reactions shown in Schemes 3.7 – 3.10.



Scheme 3.7. Reagents and Conditions: (i) Amino acid alkene (2 equiv), Grubbs second generation catalyst **1.17** (20 mol%), DCM, argon flow, reflux 6 h, then DMSO (50 equiv relative to **1.17**), rt, 12 h (**3.22/3.22a/3.22b**, 20:4:1, 86%), (**3.23**, 62%).

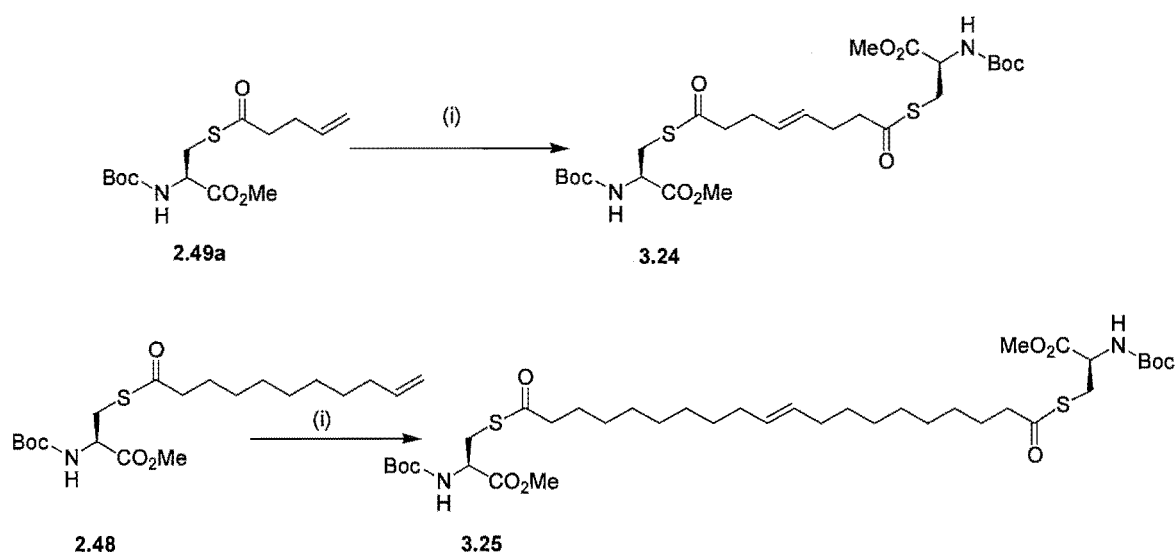
The entire structure of the trace isomers (**3.22a**, **b**) was not assigned as these compounds could not be purified from the product mixture. However, the HRMS of the **3.22/3.22a-b**

* Refer to section 2.3.3 for a discussion on double bond migrated product in CM

product mixture contained a minor M+H peak corresponding to one less methylene unit compared to **3.22**, indicating the presence of a chain contracted product, likely as result of double bond migration in substrate **2.45** prior to CM.

O-Decenoyl serine **2.46**, prepared as in Scheme 2.10, was also dimerized by treatment with catalyst **1.17**, to give **3.23** in 62% yield, which is an example of a longer cross-linked tether length compared to **3.22**. The ^1H NMR spectrum of **3.23** showed evidence of only one compound, however the HRMS revealed two minor M+H peaks that corresponded to one and two less methylene units compared to **3.23**, indicating that traces of chain contracted CM products were present.

The symmetrical dimers **3.24** and **3.25** were similarly prepared but from modified cysteine amino acids. *N*-Boc-*S*-pentenoyl-Cys-OMe **2.49a**, prepared as in Scheme 2.11, Chapter 2, was dimerized by treatment with catalyst **1.17** to afford cysteine dimer **3.24** in 45% yield (Scheme 3.8). Substrate **2.48**, containing an *S*-decenoyl tether length (Scheme 2.11), was also treated with catalyst **1.17** to give symmetrical dimer **3.25** (75%) as a mixture which contained traces of chain contracted isomers. The presence of these isomers was observed by two minor peaks in the HRMS of **3.25** that corresponded to one and two less methylene units compared to **3.25**.



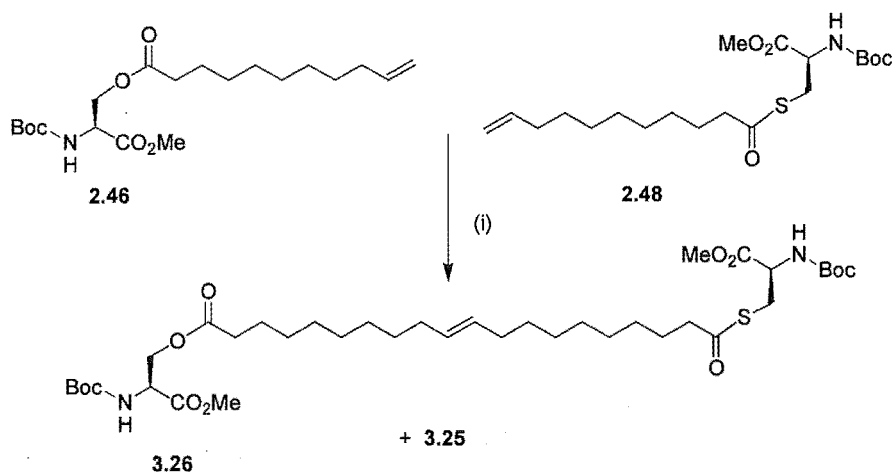
Scheme 3.8. Reagents and Conditions: (i) Amino acid alkene (2 equiv), Grubbs second generation catalyst **1.17** (20 mol%), DCM, argon flow, reflux 6 h, then DMSO (50 equiv relative to **1.17**), rt, 12 h (**3.24**, 45%), (**3.25**, 73%).

The serine and cysteine symmetrical dimers shown in Schemes 3.7 and 3.8 were all isolated in moderate to good yields. Dimers **3.22** - **3.25** were assigned as the more thermodynamically stable *E* configuration, since olefin metathesis reactions with catalyst **1.17** are presumed to be under thermodynamic control. The ^1H and ^{13}C NMR spectra of symmetrical dimers **3.22** - **3.25** showed the protons and carbons on each side of the double bond to have equivalent resonances.

Both **3.22** and **3.23** represent examples of novel side-chain to side-chain cross-linked serine amino acids. The methodology used to prepare **3.22** and **3.23** is amenable to variety of acyl tethers appended to the side chain of serine. These variable tethers can be installed using the same general acylation reaction, compared to *O*-allyl tethers of dimers such as **1.63a** (Figure 3.2) that can not so easily be varied. Symmetrical dimers **3.24** and **3.25** represent novel cysteine side-chain to side-chain tethered amino acids. Cysteine commonly forms disulfide bonds in biological systems via the side-chain thiol group. Dimers such as **3.24** and **3.25** can be considered disulfide mimics, although they are of longer tether length than native disulfide linkages. The *C-C* linkage used in this study to dimerise modified cysteine amino acids would be more stable in biological systems than a disulfide bond that can be readily reduced and cleaved.

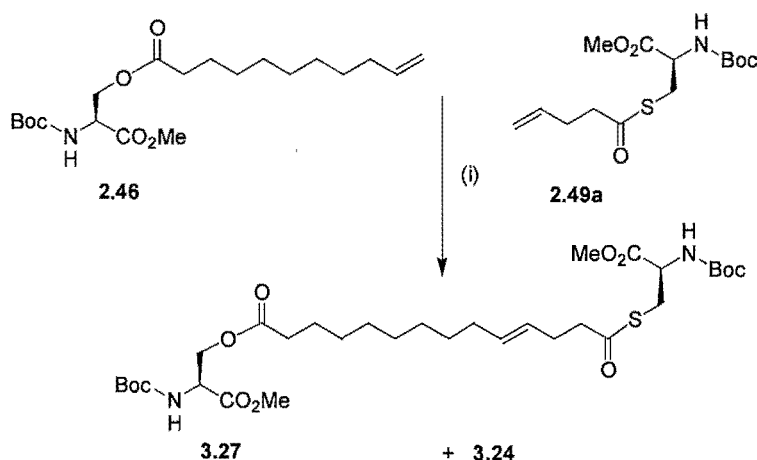
3.2.2 Unsymmetrical Dimers

Unsymmetrical dimers were also synthesised via CM, by the coupling of two different amino acid-based substrates that were suitably functionalised with an olefin group. *O*-Decenoyl serine **2.46** was coupled to *S*-decenoyl cysteine **2.48** upon treatment with catalyst **1.17** to give heterodimer **3.26** in 66% yield (Scheme 3.9). Cysteine **2.48** was used in two-fold excess, and consequently homodimer **3.25** was also isolated from the reaction mixture.



Scheme 3.9. *Reagents and Conditions:* (i) Grubbs second generation catalyst **1.17** (20 mol%), DCM, argon flow, reflux 6 h, then DMSO (50 equiv relative to **1.17**), rt, 12 h (66%).

An unsymmetrical dimer (**3.27**) was also synthesised, by the CM coupling between two substrates that each contained a different olefin tether length. *O*-Decenoyl serine **2.46** was coupled to *S*-pentenoyl cysteine **2.49a** to give dimer **3.27** in 51% yield (Scheme 3.10). Cysteine-based **2.49a** was used in two-fold excess, and consequently homodimer **3.24** was also isolated from the crude reaction mixture.



Scheme 3.10. *Reagents and Conditions:* (i) Grubbs second generation catalyst **1.17** (20 mol%), DCM, argon flow, reflux 6 h, then DMSO (50 equiv relative to **1.17**), rt, 12 h (51%).

Unlike symmetrical dimers **3.22** - **3.25**, the ^1H and ^{13}C NMR spectra of unsymmetrical dimers **3.26** and **3.27** showed non-equivalent resonances for the protons and carbons on each side of the double bond. Dimers **3.26** and **3.27** were assigned as the *E* configuration for thermodynamic reasons. A vicinal coupling constant of 15.2 Hz across the double bond was measured in the ^1H NMR spectrum of **3.27**, confirming the *E* configuration. The HRMS of both **3.26** and **3.27** showed minor M+H peaks that corresponded to one less methylene unit compared to the respective major CM product, indicating traces of chain contracted isomers were present in the product mixtures. The synthesis of unsymmetrical tether length dimers of the same type of amino acid was not attempted, as it was anticipated that separation of the CM and homodimer products would be problematic.

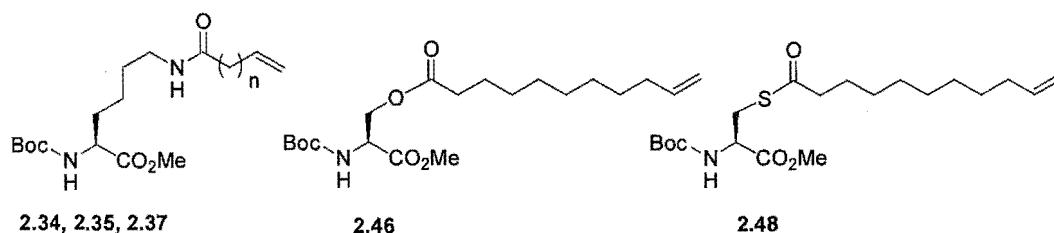
The preparation of unsymmetrical dimers **3.26** and **3.27** further demonstrates the versatility of the CM method developed in this thesis, and shows it is applicable for different types of amino acids. The general approach of amino acid side-chain acylation to install an olefin tether followed by dimerisation via CM could be also applied to other amino acids, for example tyrosine, aspartic acid, glutamic acid, or threonine.

Amino acid dimers such as **3.20** have previously been prepared by CM of acyclic precursors **3.19**, which were synthesised by acylation of the *N*-terminal amine with carboxylic acids of varying olefin tether lengths (Scheme **3.6**). Some advantages of the side-chain acylation strategy used in this thesis over this *N*-terminus method include the ability to install the olefin tether at any position along a peptide that contains an appropriate amino acid, for example serine or cysteine. *N*-Backbone amides have also been acylated with carboxylic acids containing different olefin tether lengths (**3.16**), to give dimers such as **3.17** (Scheme **3.5**). Some disadvantages of this method may include disruption to the peptide secondary structure, and that acylation of a backbone amide in a pre-assembled peptide is likely not as efficient compared to a side-chain acylation strategy.

3.2.3 Lysine Dimers

The use of modified lysine substrates **2.37**, **2.35**, and **2.34** in CM dimerisation reactions was attempted, however in all cases only the unreacted lysine substrates were recovered from the reaction. The attempted CM reactions of lysine-based substrates are outlined in Table 3.1. Initially CM reaction conditions involving a 6 h reflux period were used, but as CM coupling did not occur the reflux time was increased to 18 h in an effort to give the cross-coupled product. *N*-Pentenoyl lysine **2.35** was reacted with catalyst **1.17** under a range of reaction conditions (see Table 3.1) but an ^1H NMR spectrum of each crude reaction mixture showed only uncoupled CM substrate **2.35** (Table 3.1, entries 1, 2, and 3). The lack of dimerisation of *N*-pentenoyl lysine **2.35** was surprising given the successful RCM between the two *N*-pentenoyl tethers of both acyclic dipeptides **4.15** and **4.26** (Schemes 4.4 and 4.6, Chapter 4). Homodimer formation was then attempted with *N*-acryloyl lysine **2.37** (entry 4), but again homodimer products were not detected.

N-Acryloyl lysine **2.37** was shown in Chapter 2 to be an excellent CM substrate, and gave glyco- and lipoamino acid CM products in high yields (see Schemes 2.18, 2.20). This is thought to be because *N*_ε-acryloyl lysine **2.37** is a Type II olefin while olefins **1.51b** and **2.76** are Type I olefins, as defined in Table 1.1, Chapter 1. CM between two olefins of different Types is thought to be selective, and give higher cross-coupled product yields. Dimerisation reactions were attempted between olefin **2.37** and other lysine-based olefins such as **2.35** and **2.34**. However reaction of *N*-acryloyl **2.37** with *N*-pentenoyl lysine **2.35** (entry 5) gave only starting materials. α,β -Unsaturated amide **2.37** was also reacted with *N*-decenoyl lysine **2.34** (entry 6) but again only unreacted monomer starting materials were present in the crude reaction mixture.



Entry	Alkene One (1 equiv)		Alkene Two (2 equiv)		Reaction Conditions
	Compound	<i>n</i>	Compound	<i>n</i>	
1	2.35	2	-	-	Reflux, 6 h
2					Reflux, 18 h
3					Microwave, 6 min *
4	2.37	0	-	-	Reflux, 18 h
5	2.37	0	2.35	2	Reflux, 18 h
6	2.37	0	2.34	8	Reflux, 18 h
7	2.35	2	2.48	-	Reflux, 18 h
8	2.48	-	2.35	2	Reflux, 18 h
9	2.34	8	2.46	-	Reflux, 18 h
10	2.34	8	2.48	-	Reflux, 18 h

Table 3.1. Attempted CM dimer reactions with lysine-based substrates. Reaction conditions – Grubbs second generation catalyst **1.17** (20 mol%), DCM, argon flow, reflux (time period shown in table), then DMSO (50 equiv relative to **1.17**), rt, 12 h. * Reaction carried out under air atmosphere, catalyst **1.17** (20 mol%), DCM, 6 min in 800W microwave, then DMSO (50 equiv relative to **1.17**), rt, 12 h.

Dimer formation between lysine substrates **2.35** or **2.34** and a serine or cysteine amino acid bearing a side-chain olefin tether was then attempted. *N*-Pentenoyl lysine **2.35** was reacted with *S*-decenoyl cysteine **2.48** (entry 7) to give a mixture of **2.35** starting material and cysteine symmetrical homodimer **3.25**. CM between **2.35** and **2.48** in the opposite equivalent amounts was also attempted (entry 8), but again gave no CM cross-coupled product. Dimer formation between *N*_ε-decenoyl lysine **2.34** and another amino acid also containing an extended *N*-decenoyl tether length was attempted, to see if perhaps reduced steric clashes between the catalyst and amino acid backbone would result in cross-coupled

product formation. In separate reactions *N*-decenoyl lysine **2.34** was treated with either *O*-decenoyl serine **2.46** (entry 9) or *S*-decenoyl cysteine **2.48** (entry 10) in the presence of catalyst **1.17**, but in both cases only symmetrical serine (**3.23**) or cysteine (**3.25**) homodimers were obtained along with starting material **2.34**.

3.3 CONCLUSION AND FUTURE WORK

Amino acid dimers were prepared by CM of serine or cysteine-based substrates that contain acyl olefin tethers substituted at the side-chain. Two symmetrical serine-based- (**3.22** and **3.23**) and two cysteine-based (**3.24** and **3.25**) dimers were synthesised via CM coupling of their corresponding monomer substrates. Dimers **3.22** and **3.24** were synthesised from substrates with side-chain pentenoyl tethers, while dimers **3.23** and **3.25** were synthesised from substrates containing side-chain decenoyl tethers. Two unsymmetrical dimers (**3.26** and **3.27**) were prepared via CM coupling between a serine- and a cysteine-based substrate.

The preparation of cross-linked amino acid dimers **3.22** – **3.27** further demonstrates the versatility of the general CM methodology developed in Chapter 2. This allows for acylation of natural amino acid residues, in this case the side chains of serine and cysteine, using carboxylic acids that are of varying olefin tether length, which can be coupled via CM to give cross-linked dimeric products.

Future work could involve application of this general methodology to the preparation of target dimers designed specifically for intended biological uses. Dimers such as **3.22** – **3.27** could be used as templates to link together two bioactive peptides, resulting in an even more potent peptide dimer structure, for example as discussed in Section 3.1, Scheme 3.2. Alternatively the general methodology established in this chapter could be applied to the dimerisation of larger peptides, to create a stable C-C cross-link of desired length between two side-chain acylated amino acids, for example serine or cysteine, that contain the requisite olefin tether.

3.4 REFERENCES FOR CHAPTER THREE

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CHAPTER FOUR

RING-CLOSING METATHESIS

4.1 INTRODUCTION

RCM is now a firmly established reaction in organic synthesis. The reliability and functional group tolerance of this reaction, along with the commercial availability of catalysts such as **1.17**, has resulted in the use of RCM among organic chemists for a myriad of different applications. Some examples outlining the application of RCM to modified amino acids and peptides were discussed in Chapter 1, Section **1.6**.

The application of RCM to the construction of constrained peptidomimetics can be broadly grouped into three different categories:

- 1) The macrocyclisation of large, acyclic pre-assembled peptides that contain two suitably substituted olefin groups.
- 2) The rational design and synthesis of low molecular weight bioactive, cyclic peptidomimetic compounds specific for certain biological targets.
- 3) The synthesis of cyclic amino acid-type building blocks, which can subsequently be introduced into peptides to alter stability and/or conformational properties in a predictable way.

RCM can be carried out on pre-assembled acyclic peptides that are suitably functionalised with two olefin substituents. The requisite double bonds can be introduced after peptide synthesis or alternatively in a step-wise fashion using acyclic olefinic amino acid building blocks. RCM of suitably modified pre-assembled peptides has been used to construct β -turn mimetics, for example to generate β -turn motifs as a basis of HIV-I protease inhibition (Scheme **1.16**, Chapter 1).¹ Disulfide bond mimics have been synthesised using RCM, to provide a stable cyclic structural analogue of the natural disulfide-linked cyclic peptide. For example both single disulfide bonds (Scheme **1.27**)² and thio ether ‘knots’ of more than one disulfide bond (Scheme **1.26**)³ have been replaced with carbon-carbon linkages using RCM carried out on large peptide substrates.

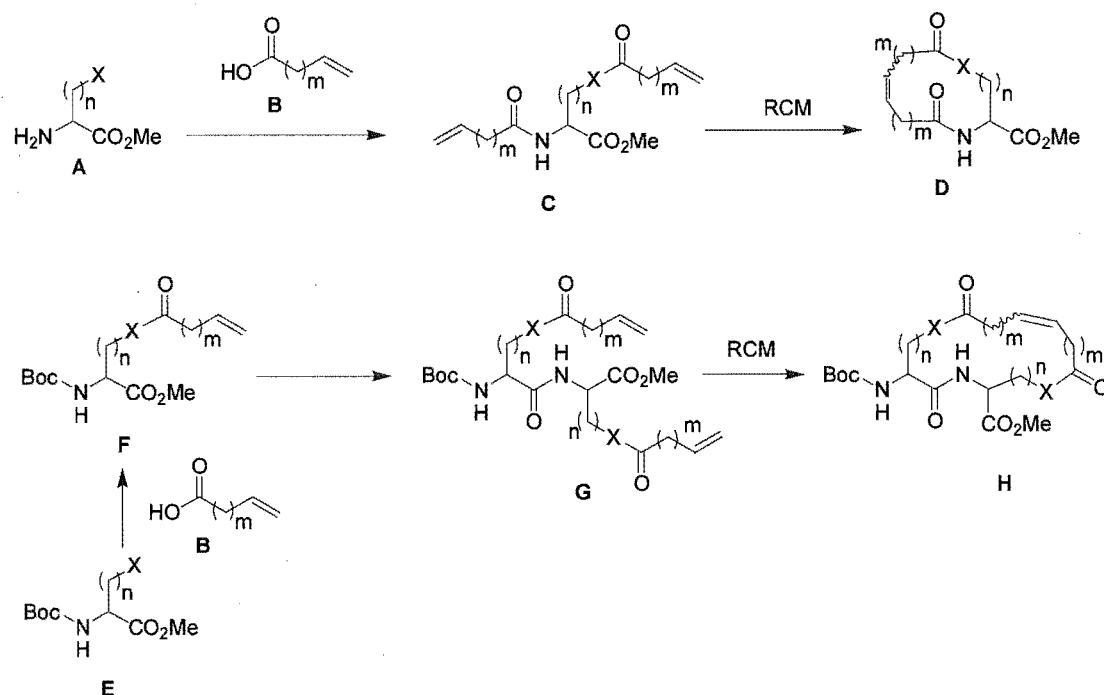
RCM has also found wide application in drug discovery where cyclic, conformationally constrained compounds prepared using this methodology have been shown to have enhanced potency relative to the corresponding acyclic analogues. This is likely due to

increased metabolic stability and structural pre-organisation into a favourable binding conformation. This work has found application in inhibition of enzymes such as HIV protease,⁴ hepatitis C virus NS3 protease,⁵ Grb2 SH2 domain-binding peptides,⁶ thrombin,⁷ and peptide deformylase.^{8,9} Constrained cyclic enzyme inhibitors provide a basis for biomechanistic investigation as well as probing structural requirements for efficient ligand-receptor interactions.

The synthesis of cyclic amino acid building blocks has attracted much attention over the years for a number of reasons. Structural motifs of this type are often found in complex natural products and peptides. For example the synthesis of small cyclic β -amino acid subunits has been studied with this application in mind (e.g. Scheme 1.19, Section 1.6).¹⁰ The incorporation of larger cyclic amino acid building blocks into peptides or peptidomimetics can induce conformational restrictions and provide important structural effects, for example α,α -disubstituted cyclic glycine derivatives have been incorporated into helical peptides (Scheme 1.22, Chapter 1).¹¹

The short, versatile, and reliable synthesis of novel, cyclic constrained amino acids-based compounds that can be used as building blocks in peptide synthesis is of ongoing interest and importance. In this thesis cyclic compounds based on lysine, serine, and cysteine amino acids and dipeptides were synthesised, resulting in a series of novel RCM products, as summarised in Figure 4.1. *C*-Terminal protected natural amino acids (**A**) were modified to contain side-chain and *N*-terminus olefin tethers by acylation with carboxylic acids (**B**) to give acyclic amino acid-based RCM precursors (**C**). The modified amino acids containing two substituted olefin tethers (**C**) were then cyclised via RCM with Grubbs second generation catalyst 1.17 to give cyclic compounds (**D**) of varying ring sizes. Cyclic compounds based on dipeptide scaffolds (**H**) were also synthesised. Here backbone protected natural amino acids (**E**) were side-chain acylated with a carboxylic acid of variable *C*-chain length (**B**) to give an amino acid that is substituted with one olefin tether (**F**). Compounds such as **F** were used as CM substrates in Chapters 2 and 3. The Boc and methyl ester group of **F** were each removed in separate reactions and the resulting free amine and the carboxylic acid were coupled to give dipeptides containing two side-chain olefin tethers (**G**). The acyclic dipeptides (**G**) were cyclised via RCM with 1.17 to give cyclic dipeptide-based compounds (**H**) of varying ring sizes. The olefin tether length and

hence ring size of **D** and **H** can be controlled by use of different lengths of carboxylic acids (**B**), resulting in a versatile general method.



Amino acid or dipeptide	n	X	m
Lysine	4	NH ₂	2, 8
Serine	1	OH	2, 8
Cysteine	1	SH	2, 8

Figure 4.1.

The general methodology presented here is not limited to a specific amino acid, as is demonstrated by the synthesis of cyclic, amino acid building blocks from different natural amino acids (**A** and **E**). This study was carried out to further explore the versatility, reliability, and efficiency of RCM as applied to amino acid and peptide systems. This chapter is divided into the type of amino acid used, and then further into the ring size of the RCM product.

4.2 LYSINE-BASED CYCLIC COMPOUNDS

Single amino acid-based cyclic compounds **4.6** and **4.13** were synthesised from their respective acyclic precursors **4.5** and **4.12**, which consisted of a side-chain and amino-terminus acylated lysine residue. Dipeptide-based cyclic compounds **4.16** and **4.21** were synthesised from side-chain acylated acyclic dipeptide precursors **4.15** and **4.20**. One example of a lysine-based cyclic dipeptide (**4.27**) was also synthesised via RCM of acyclic **4.26**, which contained side-chain and amino-terminus substituted olefin tethers as in **4.6** and **4.13**. The synthesis of this novel series of compounds was carried out to demonstrate the versatility of the general methodology shown in Figure 4.1, section 4.1, whereby the olefin tether length can easily be controlled to give cyclic products containing different ring sizes. These studies were also carried out to further explore RCM reactions with amino acid and peptide-based substrates. The following section is divided into single amino acid and dipeptide compounds.

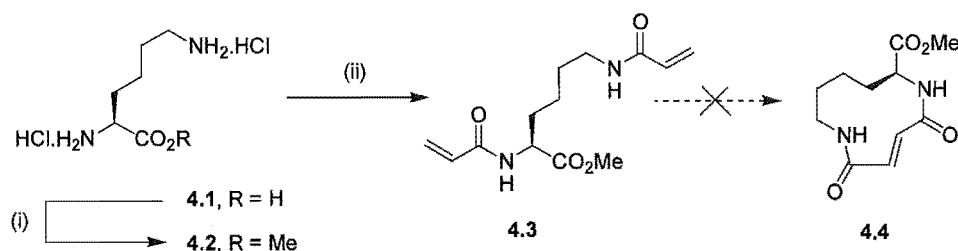
4.2.1 Lysine-based single amino acid cyclic compounds

The synthesis of three lysine-based cyclic amino acid compounds (**4.4**, **4.6**, **4.13**) was attempted by RCM of precursors containing *N*-terminus and *N*-side chain substituted olefin tethers.

Attempted synthesis of 11-membered lysine-based cyclic compound (**4.4**)

The synthesis of cyclic compound **4.4** via RCM of acyclic precursor **4.3**, which contains an α,β -unsaturated amide at both the side chain and the *N*-terminus, was attempted (Scheme 4.1). *L*-Lysine hydrochloride salt **4.1** was esterified by treatment with 2,2-dimethoxypropane and concentrated hydrochloric acid to give lysine methyl ester dihydrochloride salt **4.2**. This was then acylated upon reaction with acryloyl chloride in alkaline conditions to give *N,N*-diacryloyl **4.3** in 14% yield after silica gel-based column chromatography. The poor isolated yield of **4.3** is likely due to its relative insolubility and polymerization of its constituent α,β -unsaturated amide. *N,N*-Diacryloyl **4.3** was only

sparingly soluble in DCM, sufficient to allow characterisation by NMR spectroscopy in CDCl_3 . RCM of **4.3** using catalyst **1.17** was not attempted, as other metathesis reactions with substrates that were also only sparingly soluble in DCM, for example dipeptide-based CM substrate **2.59**, were unsuccessful. RCM was not attempted under microwave conditions due to insolubility in TCE, nor in a more polar solvent as appropriate catalysts, for example **1.20** that can be used in methanol, were not accessible. *N,N*-Diacryloyl **4.3** formed a hard, sticky solid over time, in much the same way as *N_ε*-acryloyl lysine CM substrate **2.37**.

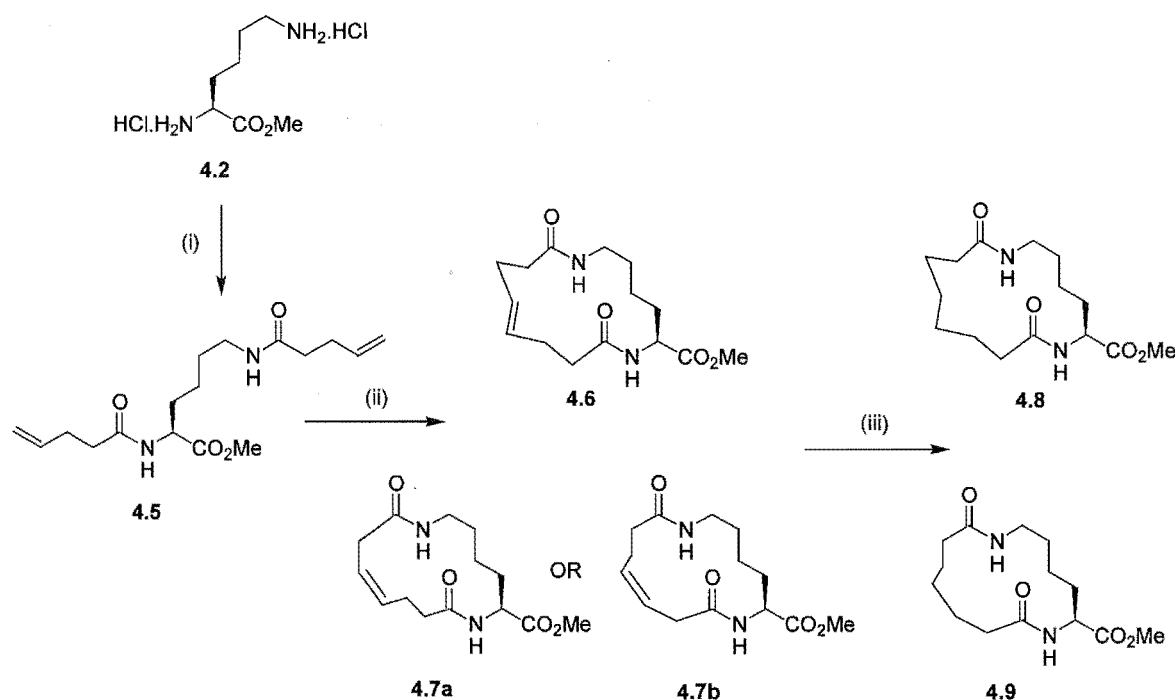


Scheme 4.1. *Reagents and conditions:* (i) 2,2-Dimethoxypropane, HCl, MeOH, reflux 2 h, then rt, 18 h (84%). (ii) Acryloyl chloride, DIPEA, DCM, 0°C, then rt, 16 h (14%).

Synthesis of 15-membered lysine-based cyclic compound (**4.6**)

A 15-membered lysine-based cyclic compound (**4.6**) was synthesised from *N_α*,*N_ε*-dipentenoyl lysine **4.5**, a suitable acyclic precursor for RCM (Scheme 4.2). *L*-Lysine methyl ester dihydrochloride salt **4.2** was acylated by treatment with 4-pentenoic acid and EDCI/HOBt to afford *N_α*,*N_ε*-dipentenoyl lysine methyl ester **4.5** in 74% yield. RCM precursor **4.5** was then cyclised using Grubbs second generation catalyst (**1.17**), in refluxing DCM, to give an inseparable mixture of cyclic products (73%), containing the *E*-15-membered **4.6** and *Z*-14-membered ring contracted compound **4.7a** or **4.7b** as a 3:1 mixture respectively. The ring-contracted product **4.7a/b** is presumed to form by double bond migration in either the *N_ε*-side chain (**4.7a**) or *N_α*-terminus (**4.7b**) pentenoyl tether of acyclic precursor **4.5**.

The mixture of the cyclic compounds **4.6** and **4.7a/b** (3:1) was hydrogenated using palladium on carbon under an atmosphere of hydrogen gas to give 15-membered **4.8** and 14-membered **4.9** in the same 3:1 ratio. This mixture was then separated by semi-preparative HPLC to give pure samples of **4.8** and **4.9**.



Scheme 4.2. *Reagents and conditions:* (i) 4-Pentenoic acid, EDCI, HOBT, DIPEA, DCM, rt, 16 h (74%). (ii) Grubbs second generation catalyst **1.17** (20 mol%), DCM, argon flow, reflux 6 h, then DMSO (50 equiv relative to **1.17**), rt, 12 h (73%, **4.6/4.7a** or **b**, 3:1). (iii) H₂, 10% Pd/C, MeOH, 18 h (quantitative, **4.8/4.9**, 3:1).

The structures of the RCM products **4.6** and **4.7a/b**, and the saturated analogues **4.8** and **4.9**, were elucidated by NMR and mass spectrometry. An HRMS of **4.8** gave an M+H parent ion peak at 299, while the ¹³C NMR spectrum of **4.8** showed fifteen distinctive peaks, both of which are in agreement with the 15-membered ring structure. An HRMS of **4.9** gave an M+H parent ion peak at 285, and fourteen distinctive resonances in the ¹³C NMR spectrum, with one less carbon between δ_C 30 – 40 ppm as compared to **4.8**, results that are consistent with the 14-membered ring contracted product (**4.7a** or **b**).

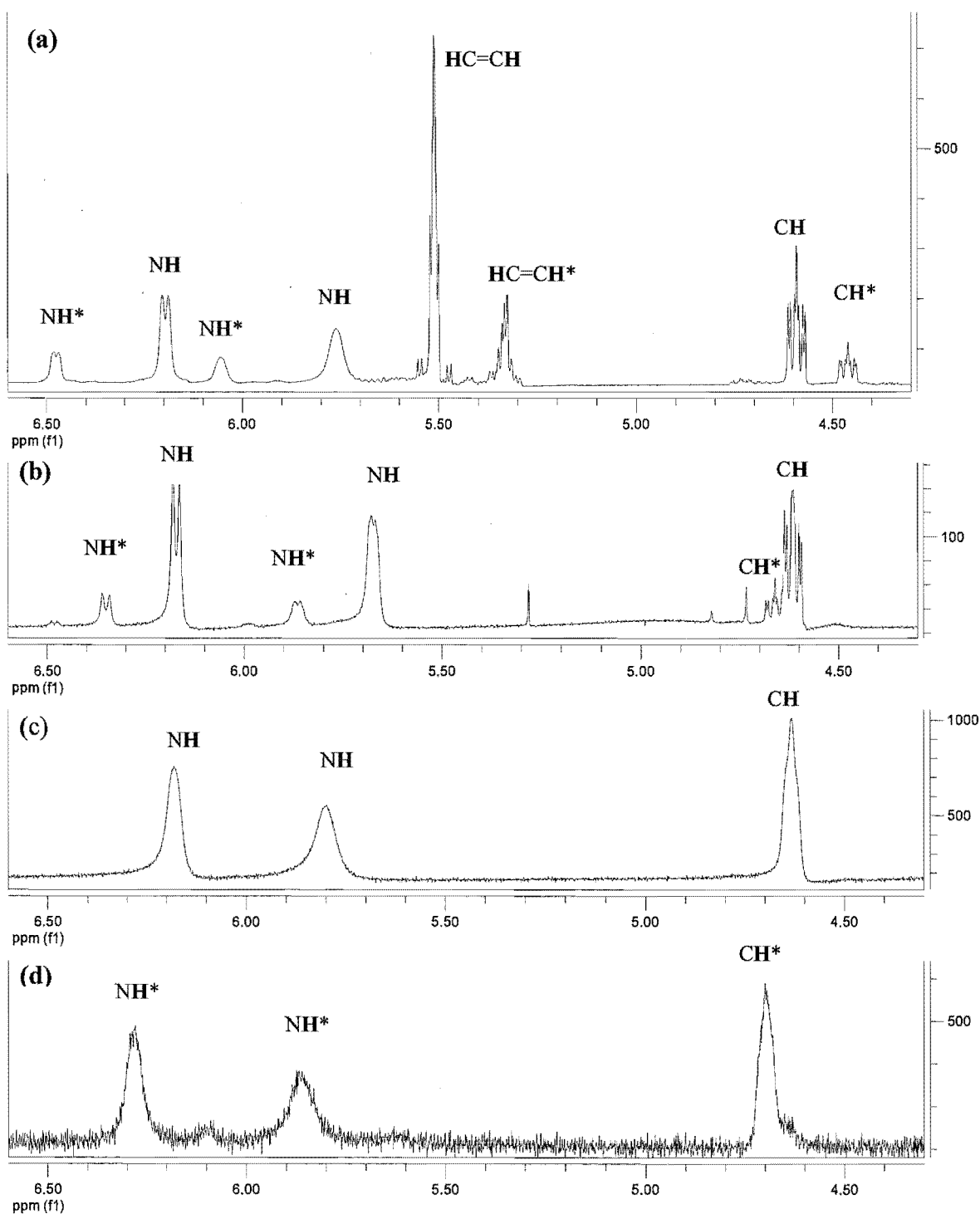


Figure 4.2. ^1H NMR spectra (500 MHz, CDCl_3) of (a) Mixture of **4.6** and **4.7a/b***, 3:1; (b) Mixture of **4.8** and **4.9***, 3:1; (c) **4.8** pure sample; (d) **4.9*** pure sample. NB. Pure samples of **4.8** and **4.9** were only sparingly soluble in CDCl_3 .

^1H NMR spectra of the mixtures of **4.6/4.7a/b** and **4.8/4.9** are shown in Figure 4.2, along with those of the pure samples of **4.8** and **4.9** for comparison. The integrals of the observed resonances that correspond to the major and minor isomers of both (**a**) and (**b**) were found to be in a 3:1 ratio. It is assumed that either **4.7a** or **4.7b** was present in the mixture and not both, as there was evidence of only two compounds in ^1H NMR spectrum (**a**), Figure 4.2, and one of these was assigned as 15-membered **4.6**.

Hydrogenation of the mixture containing **4.6/4.7a/b** gave **4.8** and **4.9** in a similar 3:1 ratio, a result which excluded the possibility of the original 3:1 mixture being due to *E* and *Z* isomerism. Peak coalescence was not observed in the high temperature NMR spectrum of **4.6/4.7a/b**, indicating the isomer mixture was not due to rotamers. A mass spectrum of the **4.6** and **4.7a/b** mixture showed $\text{M}+\text{H}$ parent ion peaks at 297 and 283. Thus the isolated mixture of products following RCM of acyclic **4.5** was assigned as the 15-membered **4.6** and 14-membered **4.7a/b**.

The ring contracted compound **4.7a/b** differs from **4.6** in that it contains an α,γ -unsaturated amide, the presence of which can be observed by a distinctive doublet at approximately δ_{H} 3.0 ppm¹ in ^1H NMR spectra. This doublet, however, was not observed in the ^1H NMR spectrum of the mixture of **4.6/4.7a/b**, due to overlapping signals. It was presumed that the resonance corresponding to the α,γ -unsaturated amide of **4.7a/b** was hidden underneath a broad multiplet pertaining to the $\text{CH}_2\text{-NH}$ protons at δ_{H} 2.95 – 3.00 ppm. A COSY of the **4.6/4.7a/b** mixture revealed coupling between this multiplet and the minor olefinic proton resonance, consistent with the presence of an α,γ -unsaturated amide.

There is strong experimental evidence to suggest that RCM using catalyst **1.17** occurs under thermodynamic control.^{12,13} Molecular modeling was carried in order to identify the most thermodynamically stable configurations of the RCM products shown in Scheme 4.3, and hence assign their double bond configuration. The assumption that *E* is more stable than *Z*, as was made for CM products in Chapters 2 and 3, was not appropriate for cyclic compounds as the most stable double bond configuration can also be greatly influenced by factors such as torsional strain and/or intramolecular hydrogen bonding.

¹ Refer to Section 2.3.3 for a discussion on double bond migrated products in olefin metathesis

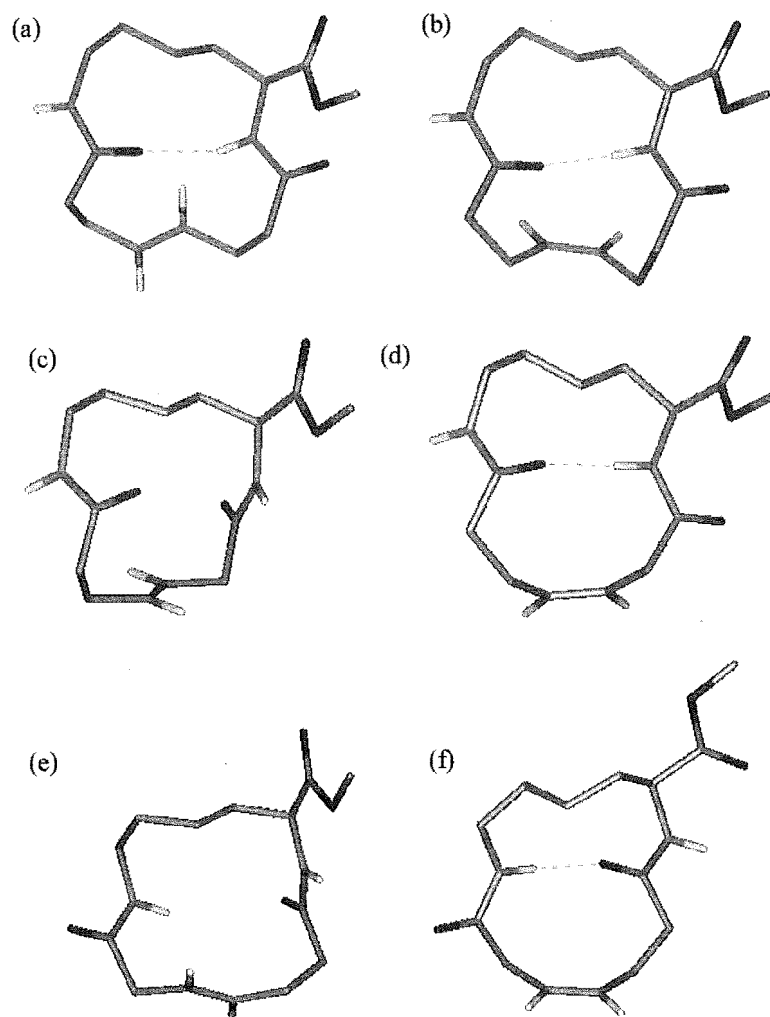


Figure 4.3. Minimized energy structures *in vacuo* of (a) *E*-4.6, -62.59 kJ/mol; (b) *Z*-4.6, -56.13 kJ/mol; (c) *E*-4.7b, 32.56 kJ/mol; (d) *Z*-4.7b, 14.91 kJ/mol; (e) *E*-4.7a, 33.29 kJ/mol; (f) *Z*-4.7a, 22.56 kJ/mol. For clarity only the amide and double bond hydrogens are shown. Boltzmann weighted average energies given.

The *E* and *Z* configurations of 4.6, 4.7a, and 4.7b were each independently constructed *in silico* using Schrödinger's Maestro build function and minimized using the MMFFs forcefield *in vacuo*, with conformations within $12 \text{ kJ mol}^{-1} \text{ \AA}^{-1}$ of the lowest energy conformation identified to generate a low energy ensemble for each of the six structures. These ensembles were then used to calculate the Boltzmann weighted average energy of each of the six configurations (*E*- and *Z*-4.6, 4.7a and b). These calculations gave *E*-4.6 to be lower in energy compared to *Z*-4.6, but *E*-4.7a and b to be higher in energy compared to

the corresponding *Z* configurations. Therefore the RCM products shown in Scheme 4.2 were assigned *E*-4.6 and *Z*-4.7a/b (Figure 4.3, lowest energy structures shown).

A hydrogen bond that spanned the ring scaffold was observed in the lowest energy structures both *E*- and *Z*-4.6. The lowest energy structures of the *Z* configuration for both ring contracted compounds (4.7a and b) also exhibited a hydrogen bond between the same atoms, while *E*-4.7a and b lacked a hydrogen bond. This is likely the reason for the greater thermodynamic stability of *Z*-4.7a and b compared to the respective *E* configurations.

RCM of acyclic diene 4.5 was also carried out under microwave conditions to provide a comparison to the standard refluxing DCM conditions discussed above. A solution of diene 4.5 and catalyst 1.17 (same molarity % as DCM conditions) in TCE was heated for six min at 800 W in a microwave. The higher boiling point of TCE (compared to DCM) makes this solvent better suited to microwave conditions. Purification of the crude reaction mixture by silica gel-based column chromatography gave an inseparable 4:1 mixture of 4.6/4.7a/b in a total yield of 70%. This mixture was then hydrogenated to give a product mixture containing 4.8/4.9 in the same 4:1 ratio.

The formation of 14-membered ring-contracted product 4.7a/b presumably occurs due to double bond migration in acyclic 4.5 prior to RCM. The formation of a smaller proportion of 4.7a/b under microwave conditions, compared to DCM reflux conditions, suggests that in this case perhaps RCM of diene 4.5 occurred at a proportionally faster rate than double bond migration of 4.5, which may have been a reflection of less ruthenium hydride species present. These kinetic factors refer to the relative rate of the competing reactions of RCM and double bond migration, and must not be confused with the thermodynamically controlled product outcome of the RCM reaction.

The structure of the minimum energy conformation of 15-membered *E*-4.6 closely resembles that of cyclic dipeptide 4.10, based on a glutamine to lysine side-chain cyclisation, that is present at the terminus of renin enzyme inhibitor 4.11 (Figure 4.4).¹⁴ The 15-membered cyclic compound 4.10 is an important component of inhibitor 4.11, evidenced by the fact that the analogues linear peptide with no cyclisation between glutamine and lysine showed a several fold lower inhibition compared to 4.11.

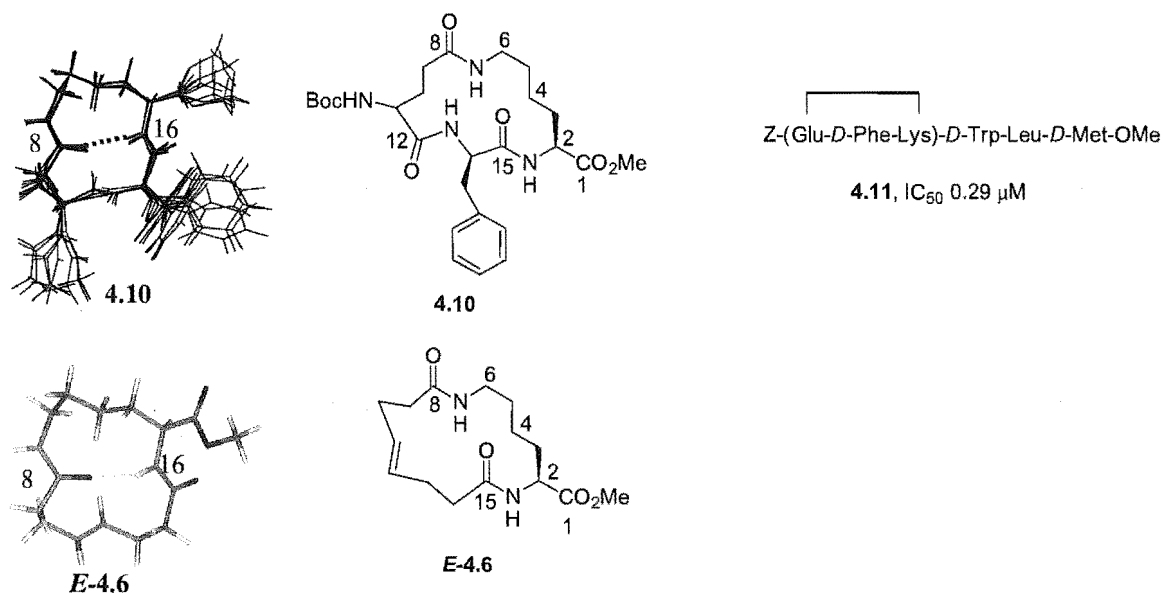


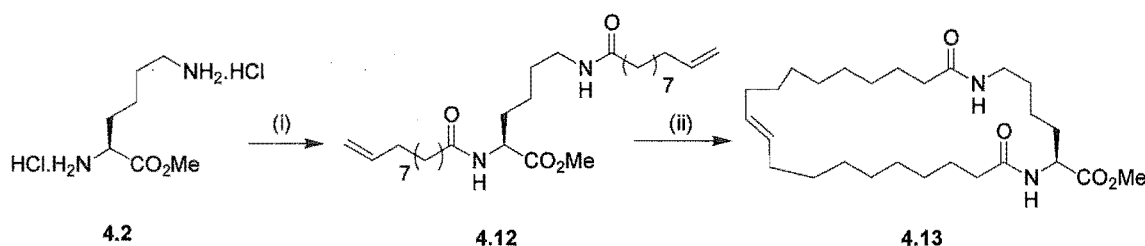
Figure 4.4. Comparison of *E*-4.6 to cyclic peptide **4.10**, a component of renin inhibitor **4.11**. Representation of **4.10** shows superimposed minimum energy structures of various conformations (taken from Kunwar *et al.*).¹⁴ Ring systems are numbered according to IUPAC naming system for **4.6**.

In addition to having the same ring size, *E*-4.6 and **4.10** both contain amide groups at ring positions 7 and 16, and carbonyl groups at ring positions 8 and 15. These two exposed carbonyl groups of **4.10** are thought to hydrogen bond to residues in the renin enzyme active site. Both *E*-4.6 and **4.10** show a similar intramolecular hydrogen bond between the amide hydrogen at position 16 and the carbonyl at position 8, resulting in a 10-membered pseudo β -turn-like cyclic structure. These similarities demonstrate that constrained, cyclic amino acid-based scaffolds such as *E*-4.6 could be incorporated into peptides to impart enhanced biological activities specific for certain therapeutic targets.

Synthesis of 27-membered lysine-based cyclic compound (**4.13**)

The synthesis of a 27-membered lysine-based cyclic compound (**4.13**) was also carried out to further demonstrate the versatility of the general RCM methodology developed in this chapter (refer to Figure 4.1, section 4.1). *L*-Lysine methyl ester dihydrochloride salt **4.2**

was acylated upon treatment with 10-decenoic and EDCI/HOBt to give N_{α},N_{ϵ} -didecenoyl lysine methyl ester **4.12** in an excellent yield of 92% (Scheme 4.3). Acyclic RCM precursor **4.12** was treated with Grubbs second generation catalyst **1.17** and the product purified by silica gel-based chromatography to give the 27-membered cyclic compound *E*-**4.13** (20%), which contained traces of isomers, including two ring-contracted products.



Scheme 4.3. *Reagents and conditions:* (i) 10-Decenoic acid, EDCI, HOBt, DIPEA, DCM, rt, 16 h (92%). (ii) Grubbs second generation catalyst **1.17**, DCM, argon flow, reflux 6 h, then DMSO (50 equiv relative to **1.17**), rt, 12 h (20%).

Molecular modeling was carried out to identify which double bond configuration of **4.13** was more thermodynamically stable. Cyclic *E*-**4.13** and *Z*-**4.13** were constructed separately in silico using Schrödinger's Maestro build function and minimized using the MMFFs forcefield *in vacuo*, to generate a low energy ensemble for each of the two structures. These ensembles were then used to calculate a Boltzmann weighted average energy for each of *E*-**4.13** and *Z*-**4.13**, which revealed *E*-**4.13** to be lower than *Z*-**4.13** (Figure 4.5, lowest energy structures shown), therefore the double bond configuration of RCM product **4.13** was assigned as *E*. A hydrogen bond was observed in the energy minimized structures of both *E*- and *Z*-**4.13**, however it was between different sets of NH-OC atoms, as shown in Figure 4.5. Structure *E*-**4.13** was hydrogen bonded through the *N*-terminus amide and side-chain carbonyl, while *Z*-**4.13** between the *N*-terminus carbonyl and side-chain amide group. The structure of *E*-**4.13** has a comparatively planar ring structure with less torsional strain compared to the twisted ring structure of *Z*-**4.13**.

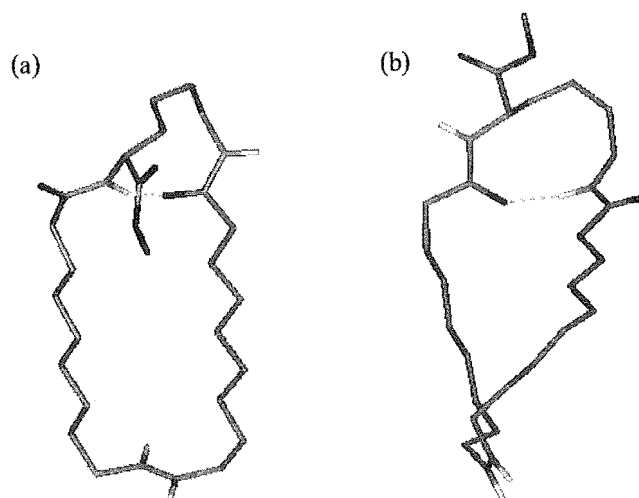


Figure 4.5. Minimized energy structures *in vacuo* of **4.13** (a) *E*, -78.78 kJ/mol, and (b) *Z*, -68.67 kJ/mol. Boltzmann weighted average energies given.

The trace isomers could not be purified from the product mixture by silica gel-based column chromatography, and as such their precise structure was not determined. The HRMS of **4.13** containing these isomers did, however, show two minor peaks, corresponding to a molecular mass with one and two less methylene units compared to **4.13**. This indicates that these structures are likely to be 26- and 25-membered ring contracted products, presumably formed as a result of double bond migration in the acyclic RCM precursor **4.12**.

Amino acid building blocks with large hydrophobic rings have been coupled to the backbone terminus of peptides to alter the structural properties of the parent peptide, as discussed in Chapter 1, Scheme 1.23.¹¹ Lysine-based **4.13** also contains a large, hydrophobic ring, and could be incorporated to the *N*-terminus of a peptide to study the changes in structural properties of the parent peptide.

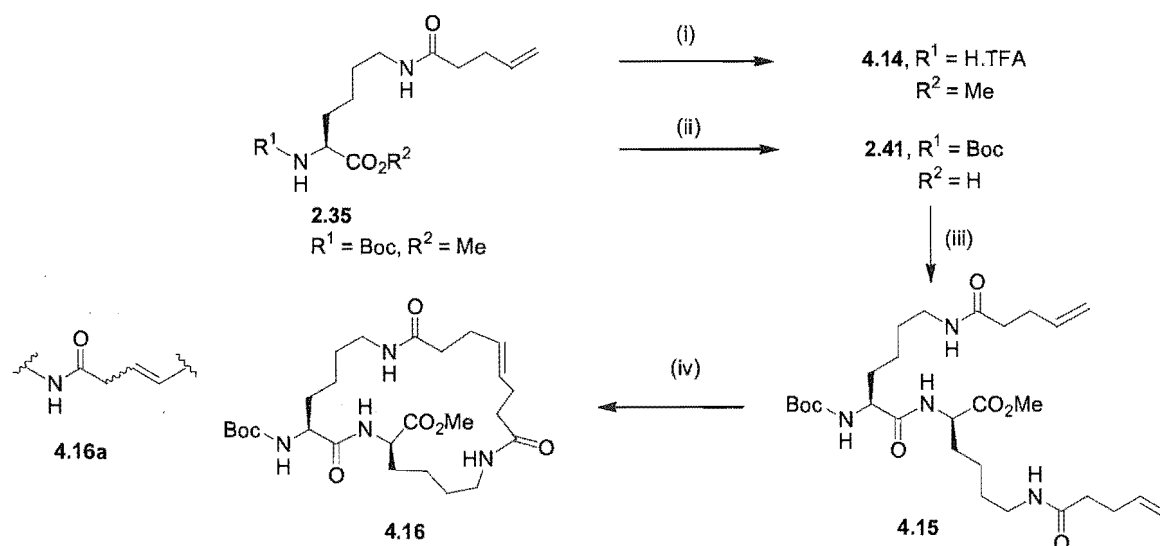
4.2.2 Lysine-based dipeptide cyclic compounds

Lysine-based cyclic dipeptides **4.16**, **4.21**, and **4.27** were synthesised to extend the scope of this general methodology beyond that of single amino acid-based compounds (refer to Figure 4.1, section 4.1).

Synthesis of 22-membered lysine-based cyclic compound (**4.16**)

*N*_α-Boc-*N*_ε-pentenoyl-*L*-lysine methyl ester **2.35** (Section 2.2, Scheme 2.8) was used as the starting material for the synthesis of 22-membered lysine-based cyclic compound **4.16**. Lysine derivative **2.35** was treated with TFA in DCM to give Boc-deprotected amine salt **4.14** (Scheme 4.4). C-Terminal deprotection of **2.35** was also carried out, where the methyl ester of **2.35** was hydrolysed upon treatment with aqueous lithium hydroxide to afford carboxylic acid **2.41**. The amine salt **4.14** and the carboxylic acid **2.41** were then coupled using HATU to give dipeptide **4.15** in 54% yield over 3 steps from **2.35**. RCM of diene **4.15** by treatment with Grubbs second generation catalyst **1.17** followed by product purification using silica gel-based column chromatography gave an inseparable mixture (30%) of *E*-**4.16** and a double bond migrated isomer (**4.16a**) (9:1 by ¹H NMR). Dipeptide-based *E*-**4.16** was isolated in 30% yield, despite the presence of only coupled CHR=CHR olefinic signals in an ¹H NMR spectrum of the crude reaction mixture following RCM. This low yield is likely the result of difficulties in isolation of the relatively polar *E*-**4.16** by silica gel-based column chromatography.

Molecular modeling of *E*-**4.16** and *Z*-**4.16** was again carried out using Schrödinger's Maestro, and revealed *E*-**4.16** to have a lower Boltzmann weighted average energy than *Z*-**4.16** (Figure 4.6, lowest energy structures shown), therefore the RCM product was assigned as *E*-**4.16**. The lowest energy structures of both *E*- and *Z*-**4.16** showed two hydrogen bonds, but in different positions – the *E* structure showed two hydrogen bonds spanning the ring structure, while the *Z* structure showed one hydrogen bond spanning the ring and one not across the ring between two atoms of the peptide backbone.



Scheme 4.4. (i) TFA, DCM, rt, 12 h. (ii) LiOH, THF, 0°C, 30 min. (iii) HATU, DIPEA, DMF, rt, 18 h (54%, 3 steps). (iv) Grubbs second generation catalyst **1.17**, DCM, argon flow, reflux 6 h, then DMSO (50 equiv relative to **1.17**), rt, 12 h (**4.16**/**4.16a** 9:1, 30%).

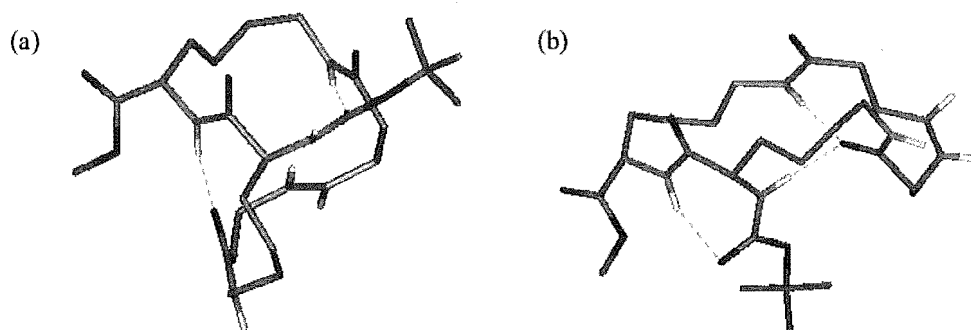


Figure 4.6. Minimized energy structures *in vacuo* of **4.16** (a) *E*, -302.08 kJ/mol; and (b) *Z*, -299.77 kJ/mol. Boltzmann weighted average energies given.

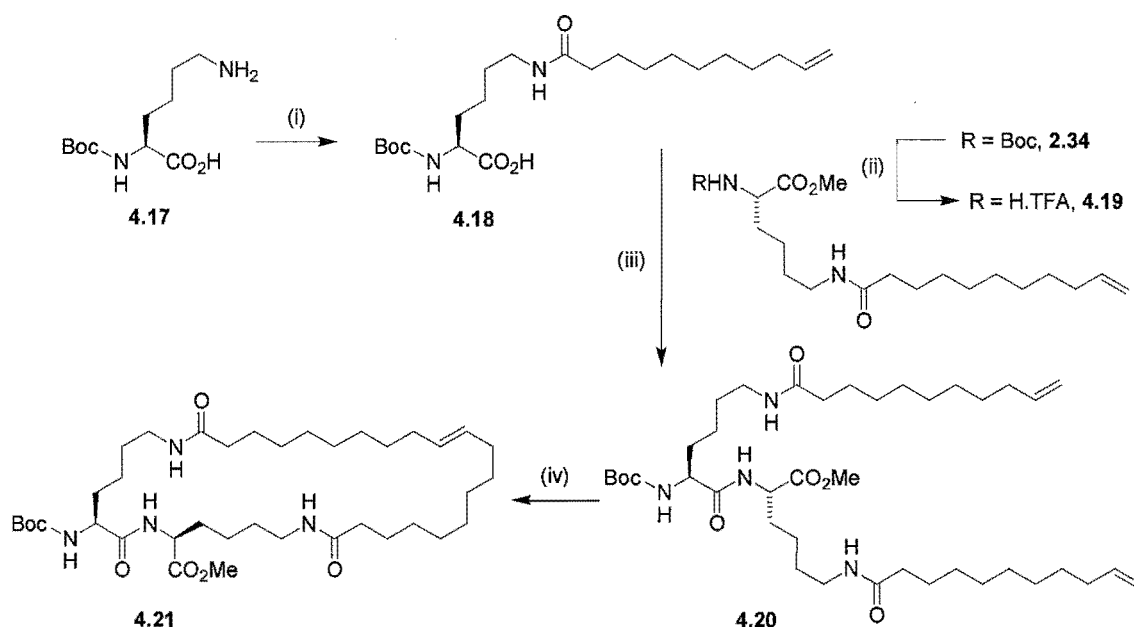
The trace isomers could not be purified by silica gel-based column chromatography, and as such their exact structure was not determined. However the ^1H NMR spectrum of **4.16** containing these isomers showed a minor doublet at δ_{H} 2.8 ppm that is consistent with an α,γ -unsaturated amide, which is presumably contained within a double bond migrated structure (**4.16a**). Isomer **4.16a** could be a ring contracted structure resulting from double bond migration in either pentenoyl tether of acyclic **4.15**, or a structure resulting from

double bond migration in 22-membered cyclic **4.16**. The HRMS of **4.16** containing traces of isomers showed a minor peak corresponding to one less methylene unit compared to **4.16**, consistent with the presence of a ring contracted product.

Compound *E*-**4.16** is an example of a cyclic, constrained amino acid building block, in that the pre-cyclised structure could be incorporated into the step-wise synthesis of a target peptide sequence, to give a modified peptide that contains a cyclic structure.

Synthesis of 34-membered lysine-based cyclic compound (**4.21**)

A 34-membered cyclic compound (**4.21**) was also prepared by RCM of an acyclic lysine-based dipeptide (**4.20**), containing two side-chain substituted *N*-decenoyl tethers (Scheme 4.5).



Scheme 4.5. (i) 10-Decenoyl chloride, NaOH, acetonitrile (64%). (ii) TFA, DCM, rt, 12 h. (iii) HATU, DIPEA, DMF, rt (87%, 2 steps). (iv) Grubbs second generation catalyst **1.17**, DCM, argon flow, reflux 6 h, then DMSO (50 equiv relative to **1.17**), rt, 12 h (28%).

Models of both *E*-**4.21** and *Z*-**4.21** were constructed using Schrödinger's Maestro build function and minimized to generate a low energy ensemble for each configuration, in

which *E*-**4.21** was found to have a lower Boltzmann weighted average energy than that of *Z*-**4.21** (Figure 4.7, lowest energy structures shown). On this basis cyclic compound **4.21** was assigned as the *E* configuration. The lowest energy structures of both *E*- and *Z*-**4.21** (Figure 4.7) each showed two hydrogen bonds, but in different positions.

The trace isomers present as a mixture along with *E*-**4.21** could not be purified by silica gel-based column chromatography, and as such their precise structure was not determined. However the HRMS of **4.21** containing these isomers showed a minor *M*+*H* peak corresponding to one less methylene unit compared to **4.21**, indicating the presence of a 33-membered ring contracted product.

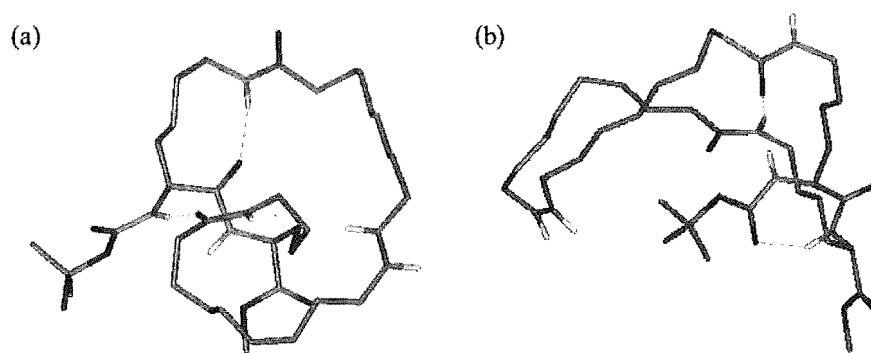
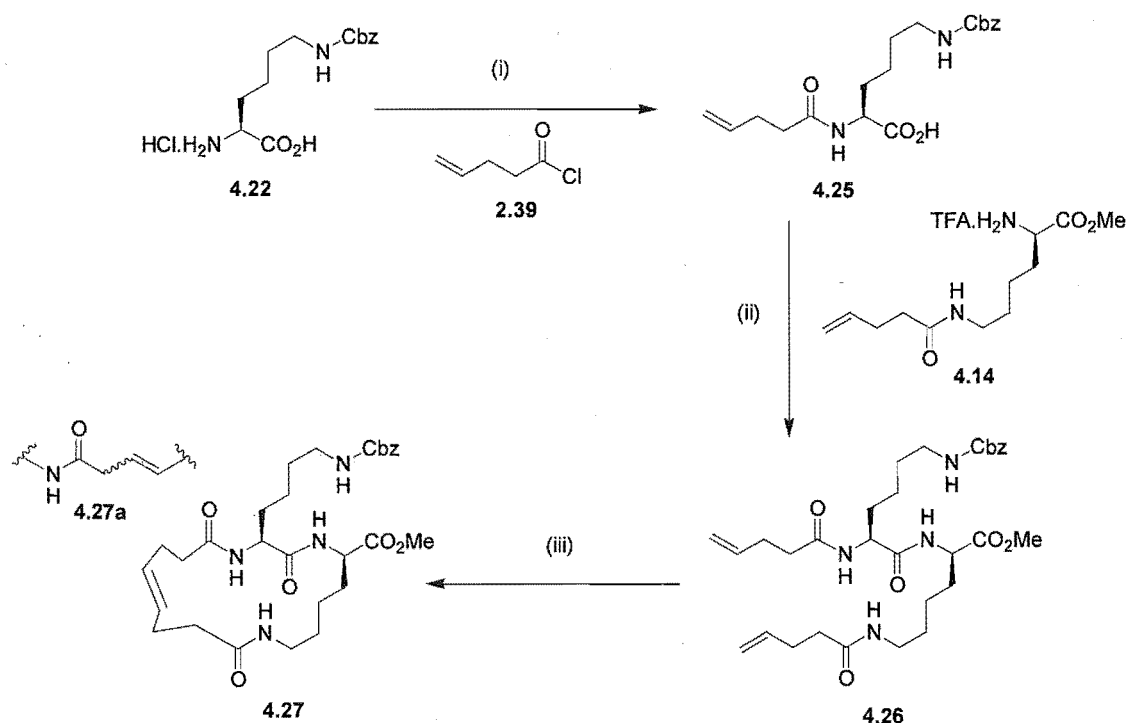


Figure 4.7. Minimized energy structures *in vacuo* of **4.21** (a) *E*, -310.38 kJ/mol, and (b) *Z*, -297.53 kJ/mol. Boltzmann weighted average energies given.

Synthesis of 18-membered lysine-based cyclic compound (**4.27**)

Cyclic dipeptide-based compounds **4.16** and **4.21** are both examples of side-chain to side-chain cyclisation products. An example of a dipeptide-based side-chain to *N*-terminus cyclic compound (**4.27**) was also constructed, via RCM of acyclic precursor **4.26** (Scheme 4.6).



Scheme 4.6. (i) 4-Pentenoyl chloride **4.24**, NaOH, acetonitrile (63%). (ii) HATU, DIPEA, DMF, rt (77%). (iii) Grubbs second generation catalyst **1.17**, DCM, argon flow, reflux 6 h, then DMSO (50 equiv relative to **1.17**), rt, 12 h (**4.27/4.27a** 8:1, 27%).

Commercially available N_ϵ -Cbz-*L*-Lys hydrochloride salt **4.22** was acylated by treatment with 4-pentenoyl chloride **2.39** (see Scheme 2.9) to give N_α -pentenoyl substituted lysine **4.25** in 63% yield. The carboxylic acid **4.25** and the amine salt **4.14** (see Scheme 4.4) were then coupled using HATU to afford dipeptide diene **4.26** in 77% yield. RCM of **4.26** was carried out using Grubbs second generation catalyst **1.17** and the product purified to give the 18-membered cyclic compound *E*-**4.27** (27%) which contained traces of isomers, including a double bond migrated isomer (**4.27a**) (8:1 by ^1H NMR).

Molecular modeling of *E*-**4.27** and *Z*-**4.27** was carried out using Schrödinger's Maestro and the Boltzmann weighted average energy was calculated for each configuration. This revealed *E*-**4.27** to be more thermodynamically stable than *Z*-**4.27** (Figure 4.8, lowest energy structures shown), therefore the RCM product **4.27** was assigned as *E*. A hydrogen bond was observed in both *E* and *Z* minimized energy structures shown in Figure 4.8, but in different positions - the *E* structure showed a hydrogen bond spanning the ring while the *Z* structure revealed weak hydrogen bonding between 3 atoms that spanned the ring.

The trace isomers could not be purified by silica gel-based chromatography, and as such their precise structure was not determined. However a minor doublet at δ_{H} 2.9 ppm was observed in the ^1H NMR spectrum of **4.27** that is consistent with a structure that contains an α,γ -unsaturated amide (**4.27a**), presumably formed due to a ring contraction and/or double bond migration. The HRMS of **4.27** containing trace isomers showed a minor $\text{M}+\text{H}$ peak corresponding to one less methylene unit compared to **4.27**, consistent with the presence of a 17-membered ring contracted product.

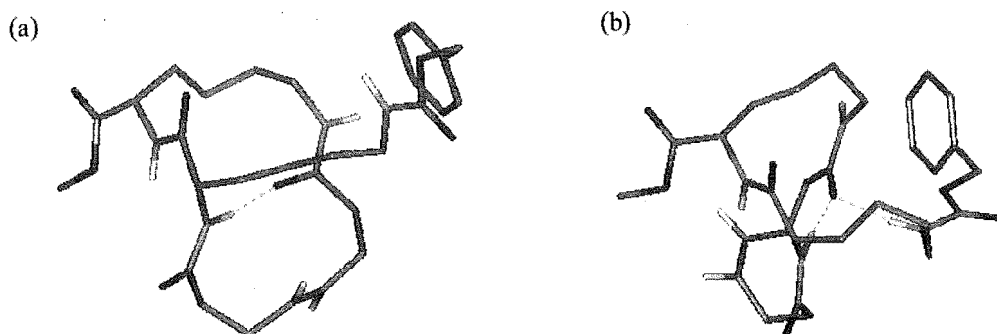


Figure 4.8. Minimized energy structures *in vacuo* of **4.27** (a) *E*, -257.45 kJ/mol, and (b) *Z*, -249.19 kJ/mol. Boltzmann weighted average energies given.

In summary, three lysine-based constrained dipeptides were synthesised – two via side-chain to side-chain cyclisation (**4.16** and **4.21**) and one via *N*-terminus to side-chain cyclisation (**4.27**). Molecular modeling of both the *E* and *Z* configurations of these cyclic structures revealed *E* to be more thermodynamically stable than *Z*, thus compounds **4.16**, **4.21**, and **4.27** were all assigned as *E*. Trace isomers were present as an inseparable mixture along with RCM products **4.16**, **4.21**, and **4.27**, of which were only assigned when they contained an α,γ -unsaturated amide (**4.16a** and **4.27a**).

The synthesis of lysine-based cyclic compounds **4.6**, **4.13**, **4.16**, **4.21** and **4.27** with varied ring size and site of olefin tether attachment was achieved using the same versatile general methodology as shown in Figure 4.1, section 4.1.

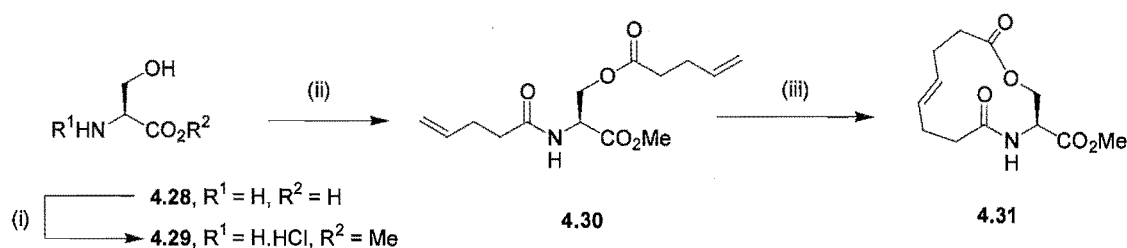
4.3 SERINE- AND CYSTEINE-BASED CYCLIC COMPOUNDS

Cyclic serine and cysteine single amino acid and dipeptide-based compounds were also synthesised in addition to the lysine-based compounds discussed in section 4.2. This was carried out to demonstrate the generality of the approach to different types of amino acids. These included three serine-based cyclic compounds (4.31, 4.33, 4.37) and four cysteine-based cyclic compounds (4.40, 4.42, 4.45, 4.46) that were all synthesised by RCM of the corresponding acyclic diene.

4.3.1 Serine-based cyclic compounds

Synthesis of 12-membered serine-based cyclic compound (4.31)

Serine-based cyclic compound 4.31 was synthesised by RCM of the acyclic precursor 4.30, which contained pentenoyl tethers substituted at the *N*-terminus and side-chain positions of serine (Scheme 4.7). *L*-Serine 4.28 was esterified by treatment with 2,2-dimethoxypropane and concentrated hydrochloric acid to give serine methyl ester hydrochloride salt 4.29. This was then acylated by treatment with 4-pentenoic acid and HATU to give *N,O*-dipentenoyl serine 4.30 in one step and in 59% yield. RCM of acyclic diene 4.30 was carried out using Grubbs second generation catalyst 1.17 to afford 12-membered serine-based cyclic *E*-4.31, the structure of which was determined by x-ray crystallography.

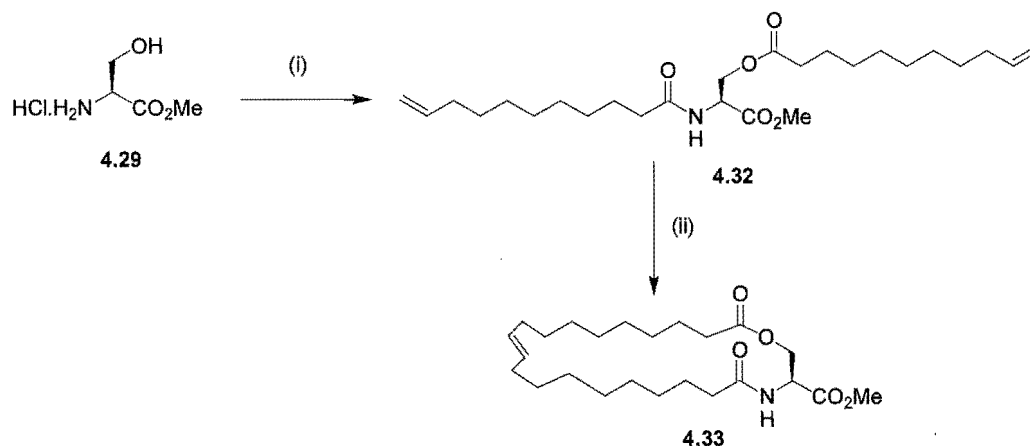


Scheme 4.7. Reagents and conditions: (i) 2,2-Dimethoxypropane, HCl, MeOH, reflux 2 h, then rt, 18 h (80%). (ii) 4-Pentenoic acid, HATU, DIPEA, DMF, rt, 16 h (59%). (iii) Grubbs second generation catalyst 1.17, DCM, argon flow, reflux 6 h, then DMSO (50 equiv relative to 1.17), rt, 12 h (45%).

The x-ray crystal structure of **4.31** revealed an *E* configuration. Molecular modeling of both *E*- and *Z*-**4.31** was carried out and compared to the x-ray crystal structure of **4.31**, as is discussed in section 4.3.4. Molecular modeling showed *E*-**4.31** to be more thermodynamically stable than the *Z*, which is in agreement with the observed *E* configuration in the x-ray crystal structure. This also validates the assignment of RCM product configurations made for other compounds in this thesis. The ^1H NMR spectrum of *E*-**4.31** showed evidence of only one compound, and the HRMS of **4.31** showed only one $\text{M}+\text{H}$ peak at 284, indicating a lack of trace isomers. Cyclic compound **4.31** has previously been reported,¹⁵ as discussed at the end of section 4.3.4.

Synthesis of 24-membered serine-based cyclic compound (**4.33**)

24-Membered cyclic compound **4.33** was synthesised by RCM of serine-based acyclic precursor **4.32** (Scheme 4.8), which contains olefin tethers substituted at the same side-chain and *N*-terminal positions as **4.30**.



Scheme 4.8. *Reagents and conditions:* (i) 10-Decenoic acid, HATU, DIPEA, DMF, rt, 16 h (54%). (ii) Grubbs second generation catalyst **1.17**, DCM, argon flow, reflux 6 h, then DMSO (50 equiv relative to **1.17**), rt, 12 h (15%).

Serine methyl ester hydrochloride salt **4.29** was acylated by treatment with 10-decenoic acid and HATU to give *N,O*-didecenoyl serine **4.32** in one step (54% yield). Acyclic precursor **4.32** was then cyclised using Grubbs second generation catalyst **1.17** and the crude product purified by silica gel-based chromatography to give 24-membered cyclic

compound *E*-4.33 (15%), which contained traces of isomers, including a ring contracted product.

The isolated yield of *E*-4.33 (15%) was much lower than that of *E*-4.31 (45%), even though ^1H NMR spectra of the crude reaction mixture in each case showed evidence of only $\text{RCH}=\text{CHR}$ coupled olefin protons. Compound 4.33 is more non-polar compared than 4.31, therefore isolation issues related to purification by silica gel-based column chromatography are presumably not a large contributing factor to the difference in isolated yields. The lower isolated yield of 4.33 could be due to substituted double bond isomers that have similar ^1H NMR resonances to product 4.33, but were not isolated from the crude reaction mixture. The yield of 4.31 may also be a reflection of comparatively less structural pre-organisation of the decenoyl tethers of acyclic 4.32 into a favourable orientation for RCM, compared to the pentenoyl tethers of acyclic 4.30.

Molecular modeling of *E*- and *Z*-4.33 was independently carried out using Schrödinger's Maestro to calculate the Boltzmann weighted average energy for each configuration (Figure 4.9, lowest energy structure shown).

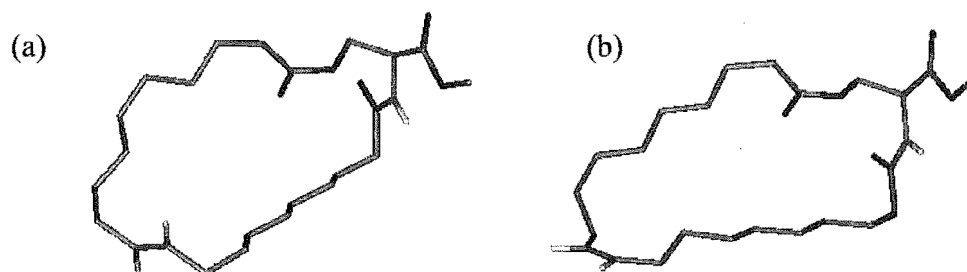


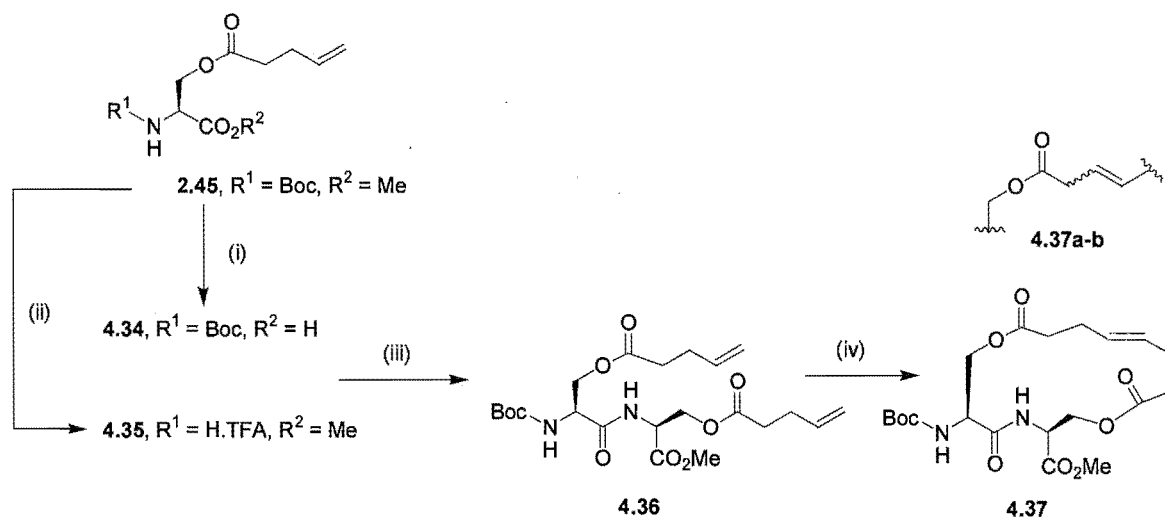
Figure 4.9. Minimized energy structures *in vacuo* of 4.33 (a) *E*, 37.14 kJ/mol, and (b) *Z*, 38.00 kJ/mol. Boltzmann weighted average energies given.

This revealed *E*-4.33 to have a lower Boltzmann weighted average energy than that of *Z*-4.33. On this basis the RCM product 4.33 was assigned as the *E* configuration. Hydrogen bond interactions were not observed in the minimized energy structures of either *E*- or *Z*-4.33. The trace isomers could not be purified using silica gel-based column chromatography, and as such their precise structure was not determined. However the HRMS of 4.33 containing these isomers showed major $\text{M}+\text{H}$ peak at 424 and a minor

M+H peak at 410, indicating the presence of a structure that contains one less methylene unit compared to **4.33**. This observed isomer was presumed to be a 23-membered ring-contracted product, resulting from double bond migration in acyclic RCM precursor **4.32**.

Synthesis of 16-membered serine-based cyclic compound (**4.37**)

Both 12-membered *E*-**4.31** and 24-membered *E*-**4.33** are examples of cyclic compounds based on a single serine residue. A cyclic compound (**4.37**), based on a serine dipeptide framework, was also synthesised via RCM of acyclic precursor **4.36** (Scheme 4.9). *O*-Pentenoyl serine **2.45**, synthesised for CM studies discussed in Chapter 2 (Scheme 2.10), was used as the starting material for the synthesis of **4.37**.



Scheme 4.0. (i) TFA, DCM, rt, 12 h. (ii) LiOH, THF, 0°C, 30 mins. (iii) EDCI, HOBt, DIPEA, DCM, rt, 18 h (13%, over 3 steps). (iv) Grubbs second generation catalyst **1.17**, DCM, argon flow, reflux 6 h, then DMSO (50 equiv relative to **1.17**), rt, 12 h (**4.37/4.37a/4.37b** 23:1:1, 45%).

Serine-based **2.45** was treated with TFA in DCM to give the Boc-protected amine salt **4.35**. The methyl ester of **2.45** was hydrolysed by treatment with aqueous lithium hydroxide to afford carboxylic acid **4.34**, and this was coupled to the amine salt **4.35** on treatment with EDCI/HOBt to give acyclic RCM precursor **4.36**. This was then cyclised using Grubbs second generation catalyst **1.17** and the product purified by silica gel-based

column chromatography to afford an inseparable mixture (45%) of 16-membered cyclic *E*-**4.37** and two double bond migrated isomers (**4.37a** and **b**) (23:1:1 by ^1H NMR).

The formation of dipeptide **4.36** in low yield (13% over 3 steps) was likely due to pentenoyl ester cleavage of **2.45** during treatment with aqueous lithium hydroxide. The duration of this reaction was shortened from 30 min to 10 min, but even this produced a mixture of products resulting from ester hydrolysis. A *C*-terminal protecting group that is labile under conditions in which the pentenoyl ester and amine protecting groups of **2.45** are not would potentially overcome this problem. Alternatively, a method selective for hydrolysis of the methyl ester over the pentenoyl ester would suffice. Methyl ester hydrolysis of **2.45** was attempted using milder conditions by treatment with 2% aqueous sodium carbonate in 1:1 THF/methanol, however this still gave a mixture of products. Other methods selective for the methyl ester of **2.45** were not attempted due to time constraints.

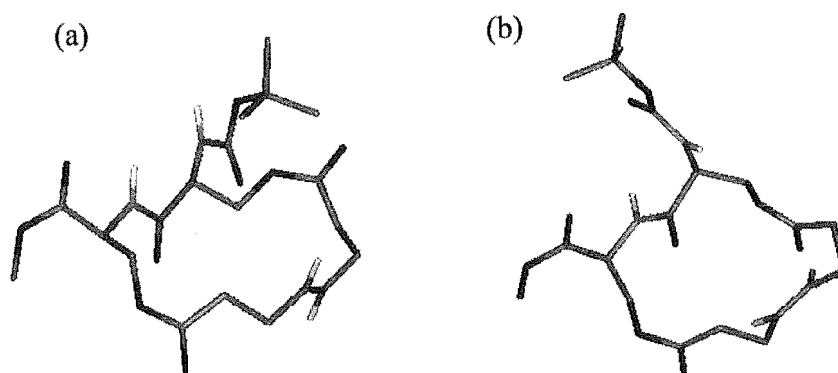


Figure 4.10. Minimized energy structures *in vacuo* of **4.37** (a) *E*, -10.04 kJ/mol, and (b) *Z*, -9.55 kJ/mol. Boltzmann weighted average energies given.

Molecular modeling of both *E*- and *Z*-**4.37** was carried out using Schrödinger's Maestro and minimized using the MMFFs forcefield *in vacuo* to generate a low energy ensemble for each configuration. From this the Boltzmann weighted average energies of *E* and *Z* were calculated, revealing *E*-**4.37** to be more thermodynamically stable than *Z*-**4.37** (Figure 4.10, lowest energy structures shown). The cyclic RCM product **4.37** was assigned as the *E* configuration on this basis. Hydrogen bonds were not observed in the lowest energy structures of *E*- or *Z*-**4.37**.

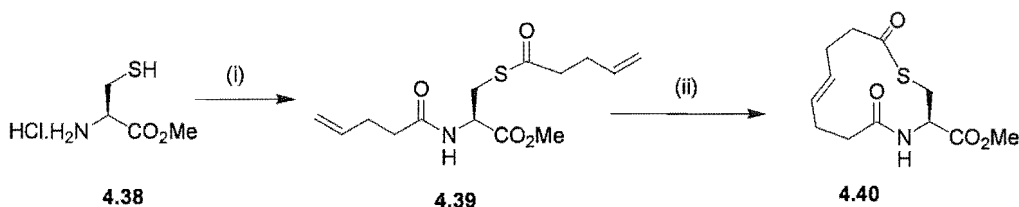
The trace isomers (**4.37a, b**) present as a mixture along with **4.37** could not be purified by silica gel-based column chromatography, and as such their entire structure was not determined. Two minor doublets at δ_{H} 2.97 and 3.03 ppm were, however, observed in the ^1H NMR spectrum of the **4.37** product mixture, consistent with isomers containing an α,γ -unsaturated amide. These are likely formed as a result of double bond migration in acyclic **4.36** and/or RCM product **4.37**. Isomer structures containing an α,γ -unsaturated amide were present along with lysine-based dipeptides **4.16** (**4.16a**) and **4.27** (**4.27a**), that were also formed during RCM of an acyclic precursor with pentenoyl tethers. The HRMS of **4.37** was consistent with these isomers (**4.37a, b**) with a minor ion corresponding to one less methylene unit compared to **4.37**, indicating the presence of a 21-membered ring contracted product.

The same isolated yields (45%) obtained for the dipeptide-based product **4.37** and single amino acid-based **4.31**, both synthesised from acyclic precursors that contained the same *N*-pentenoyl tethers, demonstrates that this general methodology (see Figure 4.1, section 4.1) is equally applicable to larger molecular architectures.

4.3.2 Cysteine-based cyclic compounds

Synthesis of 12-membered cysteine-based cyclic compound (**4.40**)

A cysteine-based cyclic amino acid (**4.40**) was synthesised via RCM from an acyclic cysteine-based diene (**4.39**) that contains *S*-side chain and *N*-terminus olefin tethers, to further demonstrate the generality of this methodology (Scheme 4.1, section 4.1) to other amino acids. Commercially available *L*-cysteine methyl ester hydrochloride salt **4.38** was acylated by reaction with 4-pentenoic acid and BOP-Cl to give *N,S*-dipentenoyl cysteine methyl ester **4.39** in one step and 88% yield.

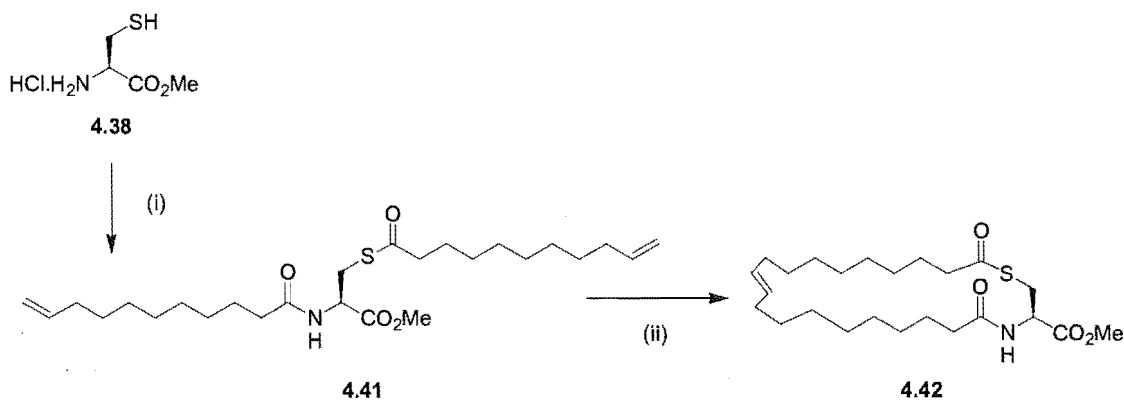


Scheme 4.10. *Reagents and conditions:* (i) 4-Pentenoic acid, BOP-Cl, TEA, DCM, 0°C, then rt, 18 h (85%). (ii) Grubbs second generation catalyst **1.17**, DCM, argon flow, reflux 6 h, then DMSO (50 equiv relative to **1.17**), rt, 12 h (74%).

RCM was carried out by treatment of **4.39** with Grubbs second generation catalyst **1.17**, to afford 12-membered cysteine-based cyclic compound *E*-**4.40** in 74% yield. RCM product **4.40** was analysed x-ray crystallography, which revealed the double configuration to be *E*. Cysteine-based *E*-**4.40** differs from serine-based *E*-**4.31** (see Scheme 4.7) by the presence of a sulfur atom derived from cysteine instead of an oxygen atom derived from serine. Molecular modeling of both *E*- and *Z*-**4.40** was carried out, and these results are discussed and compared to the crystal structure of *E*-**4.40** in section 4.3.4. As was the case for serine-based *E*-**4.31**, the molecular modeling results showed the *E* configuration to be more thermodynamically stable than *Z*, in agreement with the observed configuration x-ray crystal structure, and also further validating other molecular modeling results.

Synthesis of 24-membered cysteine-based cyclic compound (**4.42**)

In a likewise fashion to the synthesis of 24-membered serine-based cyclic compound **4.33**, cysteine-based **4.42** was synthesised via RCM from an acyclic precursor (**4.41**) that contains *N*-terminus and side-chain decenoyl tethers (Scheme 4.11). *L*-Cysteine methyl ester hydrochloride salt **4.38** was acylated by treatment with 10-decenoic acid and BOP-Cl to afford *N,S*-didecenoyl acyclic RCM precursor **4.41** in one step (48% yield). RCM of **4.103-2.1** was carried out using Grubbs second generation catalyst **1.17** and the product purified by silica gel-based column chromatography to give an inseparable mixture (43%) of *E*-**4.42** and trace isomers, including a ring-contracted product.



Scheme 4.11. *Reagents and conditions:* (i) 10-Decenoic acid, BOP-Cl, TEA, DCM, 0°C, then rt, 18 h (44%). (ii) Grubbs second generation catalyst **1.17**, DCM, argon flow, reflux 6 h, then DMSO (50 equiv relative to **1.17**), rt, 12 h (43%).

Molecular modeling of both *E*- and *Z*-**4.42** was independently carried out using Schrödinger's Maestro, and from the lowest energy ensembles that were generated the Boltzmann weighted average energy of each configuration was calculated (Figure 4.11, lowest energy structures shown). This revealed *E*-**4.42** to be lower in energy than *Z*-**4.42**, and as such the configuration of RCM product **4.42** was assigned as *E*. The lowest energy structures of *E*- and *Z*-**4.42** both showed evidence of a hydrogen bond between the thio ester carbonyl and the amide hydrogen.

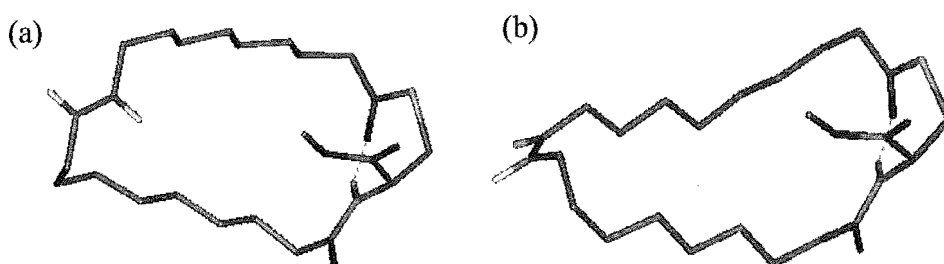


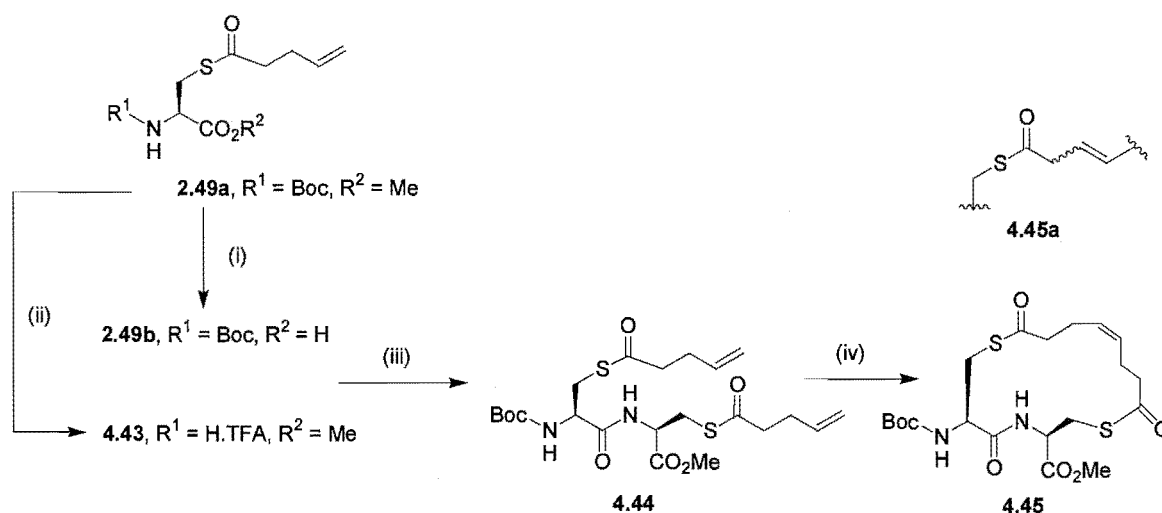
Figure 4.11. Minimized energy structures *in vacuo* of **4.42** (a) *E*, 9.65 kJ/mol, and (b) *Z*, 15.82 kJ/mol. Boltzmann weighted average energies given.

The trace isomers present in the sample of *E*-**4.42** could not be purified by silica gel-based column chromatography, and as such their precise structures were not determined. The HRMS of **4.42** that contained these isomers did, however, show a minor peak that corresponded to one less methylene unit compared to **4.42**, presumably due to the presence

of a 23-membered ring contracted product formed due to double bond migration of acyclic precursor **4.41**.

Synthesis of 16-membered cysteine-based dipeptide cyclic compound (**4.45**)

Cysteine-based cyclic dipeptide **4.45** was synthesised from acyclic dipeptide precursor **4.44**, using the same general methodology employed for the synthesis of 16-membered serine-based **4.37**, but instead using **2.49a** as the starting material. *S*-Pentenoyl cysteine **2.49a** (Scheme 2.11, Chapter 2) was treated with TFA in DCM to afford the Boc-deprotected amine salt **4.43** (Scheme 4.12). The methyl ester of **2.49a** was hydrolysed by treatment with aqueous lithium hydroxide to give carboxylic acid **2.49b**, which was then coupled to the amine salt **4.43** using HATU to give dipeptide-based RCM precursor **4.44** (11% yield over 3 steps). Diene **4.44** was cyclised via RCM using with Grubbs second generation catalyst **1.17** and the product purified by silica gel-based chromatography to give *Z*-**4.45** (36%) that contained traces of isomers, including the double bond migrated isomer **4.45a** (10:1 by ^1H NMR).



Scheme 4.12. (i) LiOH, THF, 0°C , 30 min. (ii) TFA, DCM, rt, 12 h. (iii) HATU, DIPEA, DMF, rt, 18 h (11%, over 3 steps). (iv) Grubbs second generation catalyst **1.17**, DCM, argon flow, reflux 6 h, then DMSO (50 equiv relative to **1.17**), rt, 12 h (**4.45/4.45a** 10:1, 36%).

The low yield (11%) of dipeptide **4.44** could have been due to thio ester cleavage of **2.49a** during treatment with lithium hydroxide. However, preparation of *S*-pentenoyl- β -cysteine **2.51** (Chapter 2, Scheme **2.11**) that also involved treatment of **2.49a** with aqueous lithium hydroxide, followed by further elaboration, proceeded efficiently. This indicates that minimal *S*-pentenoyl ester cleavage occurs under these conditions. As is in the case for serine dipeptide **4.36**, a different *C*-terminal protecting group strategy may be required to improve the yield of dipeptide **4.44**. Due to time constraints this was not investigated. The emphasis in this thesis was on the subsequent RCM of acyclic dipeptide-based **4.44** to give cyclic product **4.45**.

Molecular modeling using Schrödinger's Maestro and Boltzmann weighted average energy calculations was carried out independently for both *E*- and *Z*-**4.45**, which showed the *Z* configuration to be more thermodynamically stable than *E* (Figure **4.12**, minimum energy structures shown). The RCM product **4.45** was therefore assigned as *Z*. This is the only dipeptide-based compound synthesised in this thesis where the *Z* configuration was shown to be more thermodynamically stable than *E*. The minimum energy structure of *E*-**4.45** did not show any hydrogen bonding, while *Z*-**4.45** showed a hydrogen bond between the Boc group carbonyl and amide hydrogen of the adjacent cysteine residue, perhaps a reason for the lower energy of the *Z* configuration.

The exact structure of the trace isomer (**4.45a**) present as a mixture with *Z*-**4.45** was not assigned since it could not be separated by silica gel-based column chromatography. However, the α,γ -unsaturated amide of isomer **4.45a** could be assigned, as a minor doublet at δ_{H} 3.0 ppm was observed in the ^1H NMR spectrum of the product mixture. This trace isomer (**4.45a**) is presumably formed due to double bond migration in either of the *O*-pentenoyl tethers. An HRMS showed a minor *M*+*H* peak corresponding to one less methylene unit compared to **4.45**, suggesting that isomer **4.45a** is a 15-membered ring contracted product presumably formed by double bond migration in acyclic RCM precursor **4.44**. The 16-membered serine-based compound **4.37** was also isolated along with traces of an isomer containing an α,γ -unsaturated amide.

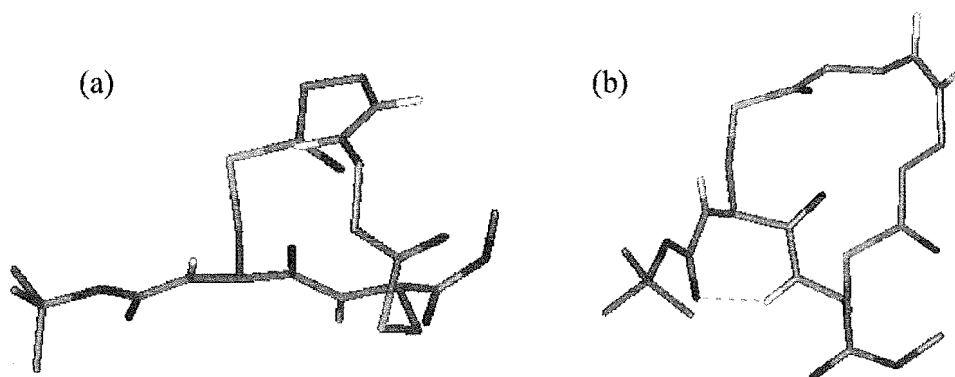


Figure 4.12. Minimized energy structures *in vacuo* of **4.45** (a) *E*, -76.76 kJ/mol, and (b) *Z*, -77.89 kJ/mol. Boltzmann weighted average energies given.

To summarize, three serine-based (**4.31**, **4.33**, **4.37**) and three cysteine-based (**4.40**, **4.42**, **4.45**) cyclic compounds were synthesised via RCM of an acyclic precursor. The acylation of natural amino acids and dipeptides with olefin-containing carboxylic acids of controllable lengths, followed by RCM to give cyclic compounds of varied ring sizes has therefore been demonstrated as a viable general method. These constrained, cyclic amino acid building block-type compounds could be incorporated into peptides to potentially alter the biological or physical properties of the parent peptide in an intended manner.

The 5 lysine-, 3 serine-, and 3 cysteine-based constrained, cyclic amino acids and dipeptides described here are interesting molecular architectures in their own right. For example this array of 11 compounds could be added to an in-house library and tested in screening assays to identify potential biological activities against intended therapeutic targets. The double bond moiety of the cyclic compounds could also be elaborated, for example by epoxidation, dihydroxylation, or bromination, to produce a library of highly functionalised novel molecular architectures.

4.3.3 Synthesis of cysteine-*tert*-leucine-based dipeptide (4.46)

The RCM products described in sections 4.1 and 4.2 are not specifically designed to target a particular biological receptor. Attempts to extend the general methodology outlined in Figure 4.1, section 4.1 to one such specific target, cyclic peptide deformylase (PDF), are described in the following section. PDF is an essential enzyme in the life cycle of all eubacteria, and thus has been identified as a therapeutic target for antibacterial drug design.¹⁶⁻¹⁸

A target compound (4.46) was synthesised based on the reported PDF inhibitor 4.47 (Figure 4.13),^{9,19} also synthesised using RCM, that shows enhanced inhibition compared to the analogous acyclic compound. Some similarities between 4.46 and 4.47 include the same ring size, and a dipeptide-based scaffold containing a *tert*-leucine residue, known to be important in inhibitor binding. Compound 4.46 does differ from 4.47 in some respects, for example it lacks a metal chelating warhead, and contains a thio ester where inhibitor 4.47 contains an unsubstituted hydrocarbon chain. The methyl ester of 4.46 could potentially be derivatised to contain a metal chelating warhead.^{8,20} In this thesis, cyclic 4.46 was synthesised by the RCM of an acyclic dipeptide-based precursor (4.52) that has pentenoyl tethers substituted at the *N*-terminus and *S*-side chain positions. The olefin tethers of RCM precursor 4.52 can be installed via an acylation reaction with a carboxylic acid, thus demonstrating the general methodology described in this thesis to a target compound.

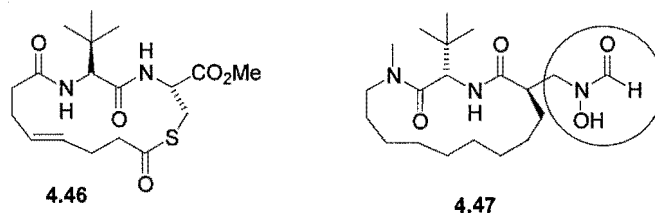
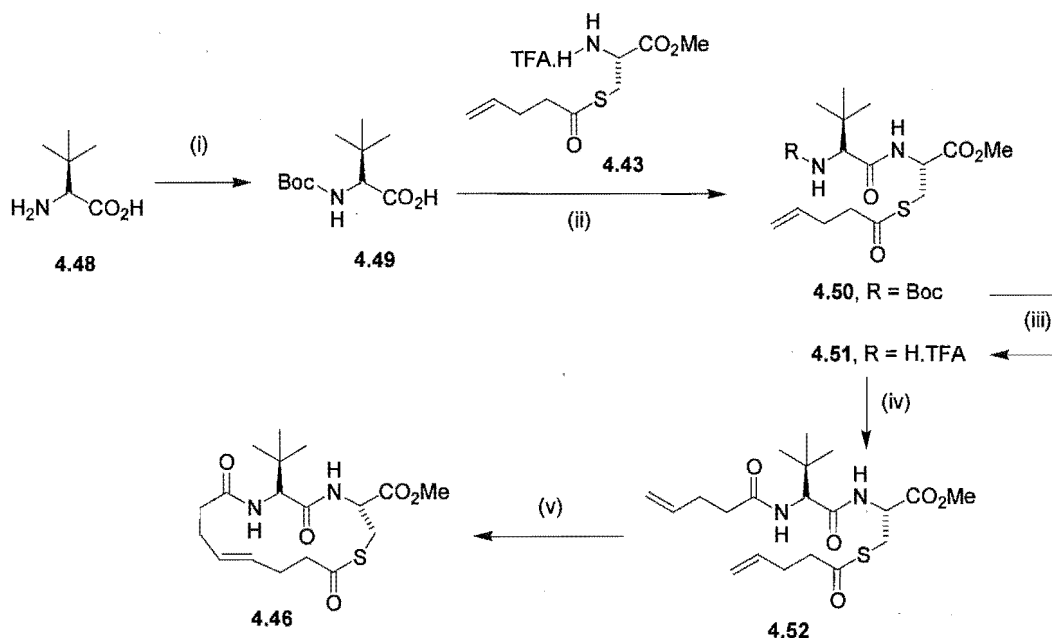


Figure 4.13. Comparison of 4.46 to PDF inhibitor 4.47.⁹ (Metal-chelating warhead circled).

Commercially available *tert*-L-leucine **4.48** was treated with Boc-anhydride in the presence of sodium hydroxide to give Boc-protected **4.49** in quantitative yield (Scheme 4.13). Boc-*L*-*tert*-Leucine **4.49** and *S*-pentenoyl cysteine methyl ester amine salt **4.43** (see Scheme 4.12) were treated with HATU in DMF, but the expected coupled dipeptide was not isolated. However reaction of **4.49** with **4.43** in the presence of EDCI/HOBt in DCM gave dipeptide **4.50** in a moderate yield of 46%. *tert*-Leucine-cysteine dipeptide **4.50** was then treated with TFA in DCM to give Boc-deprotected **4.51**, and this was acylated with 4-pentenoic using EDCI/HOBt to afford acyclic RCM precursor **4.52** in 72% yield over 2 steps. Diene **4.52** was cyclised using Grubbs second generation catalyst **1.17** to give 15-membered compound *E*-**4.46** in 65% yield.



Scheme 4.13. Reagents and Conditions: (i) Boc₂O, NaOH, water/*tert*-butyl alcohol, 18 h, rt (99%). (ii) EDCI, HOBt, DIPEA, DCM, 16 h (46%). (iii) TFA, DCM, rt, 18 h. (iv) 4-Pentenoic acid, EDCI, HOBt, DIPEA, DCM, rt, 16 h (72%, over 2 steps). (v) Grubbs second generation catalyst **1.17**, DCM, argon flow, reflux 6 h, then DMSO (50 equiv relative to **1.17**), rt, 12 h (65%).

Molecular modeling of **4.46** was carried out to determine the most thermodynamically stable double bond configuration, and therefore enable assignment of the RCM product. Both *E*- and *Z*-**4.46** were independently modeled using Schrödinger's Maestro, and the Boltzmann weighted average energy for each configuration was calculated from the

minimized energy ensemble (Figure 4.14, lowest energy structures shown). These results showed *E*-**4.46** to be more thermodynamically stable than *Z*-**4.46**, therefore the RCM product was assigned as *E*-**4.46**.

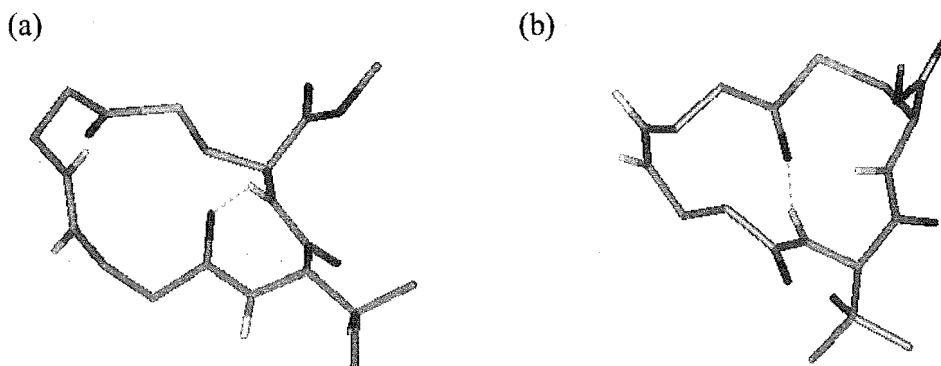


Figure 4.14. Minimized energy structures *in vacuo* of **4.46** (a) *E*, 113.26 kJ/mol, and (b) *Z*, 116.65 kJ/mol. Boltzmann weighted average energies given.

The minimized energy structures of *E*- and *Z*-**4.46** both revealed a hydrogen bond, but in the lower energy *E* conformation between the *N*-pentenoyl carbonyl and cysteine amide, and in the *Z* configuration between the thio ester carbonyl and the *tert*-leucine amide.

The synthesis of cyclic dipeptide-based **4.46** was carried to demonstrate that the general methodology involving acylation of natural amino acid functional groups followed by RCM is a viable route to the core of compounds of the type **4.47**. The versatility and control over olefin tether length discussed in this thesis is also applicable in this case, where for example cyclic compounds based on **4.46** with different ring sizes could be synthesised. This approach can be useful in the design-based synthesis of therapeutic compounds, where the optimum ring size for ligand-receptor binding can be determined through structure-activity relationships.

4.3.4 Structural analysis of 12-membered cysteine- and serine-based cyclic compounds (4.40 and 4.31)

X-Ray crystal structures

Crystal structures of cysteine-based **4.40** and serine-based **4.31** cyclic compounds were obtained to determine their structure and configuration, and were both found to be the *E* configuration (Figure 4.15).

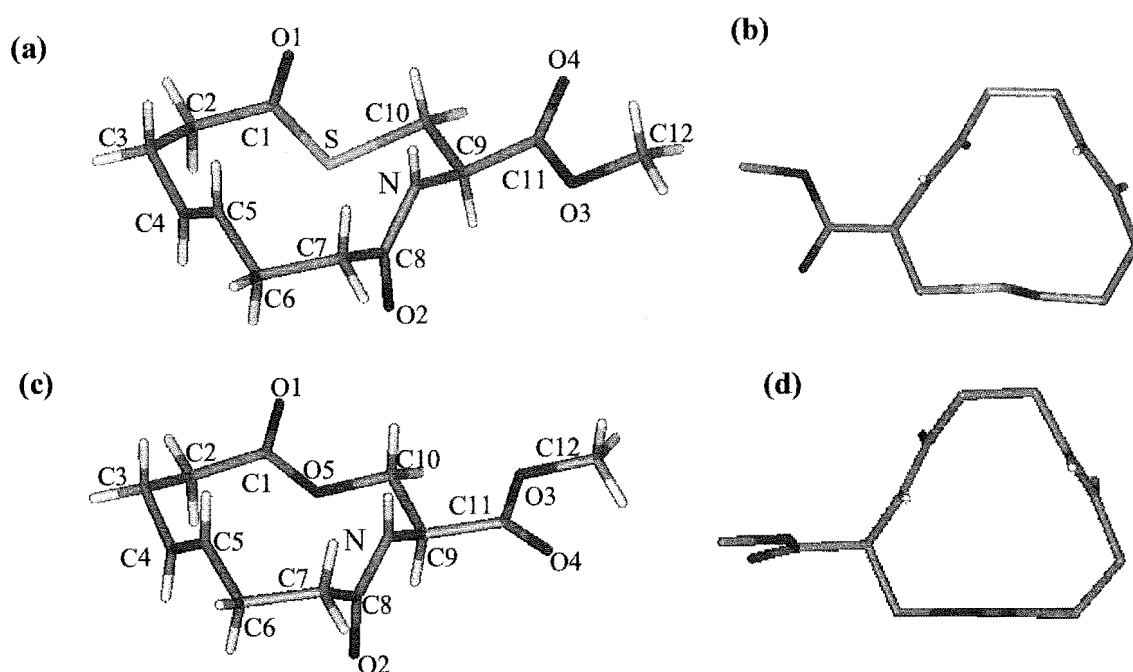


Figure 4.15. X-ray crystal structures of *E*-**4.40** (a, b) and *E*-**4.31** (c, d).

Cyclic compound *E*-**4.31** has been reported previously,¹⁵ as outlined later in this section, while *E*-**4.40** is presented for the first time in this thesis. The structures of *E*-**4.40** and *E*-**4.31** both reveal a low trans-annular and torsional strain, which can be likened to the insertion of planar, sp^2 -hybridised two-atom elements with *trans* geometry into the ring bonds of a fully hybridized six-membered ring (Figure 4.16). The ring-forming reaction in the synthesis of **4.40** and **4.31** was RCM, which installed the *E* double bond as the last *trans* element. This alternating pattern of sp^2 and sp^3 hybridized two-atom elements results

in a hexagonal shaped ring structure, which is observed in the x-ray crystal structures *E*-4.40 and *E*-4.31, as shown in Figure 4.15, (b) and (d). An intermolecular hydrogen bond was observed between the amide hydrogen and O2 oxygen of different molecules in both *E*-4.40 (2.82 Å) and *E*-4.31 (2.83 Å).

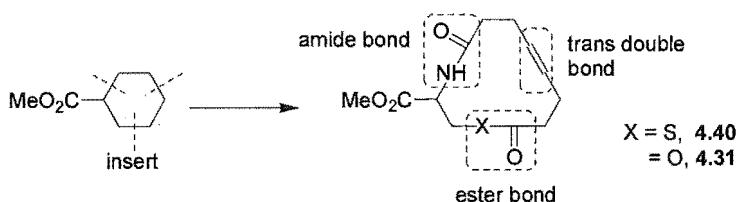


Figure 4.16. Illustration of the ideal hexagonal shaped ring structure with low torsional and trans-annular strain. Adapted from Schreiber *et al.*¹⁵

Superposition of the x-ray crystal structures *E*-4.40 and *E*-4.31 reveals some structural differences (Figure 4.17). The cyclic scaffold of each structure is very similar, with the ring portion of C9, N to C4, C3 in each case almost completely overlapping. The only difference in chemical composition between the two compounds is the presence of a sulfur atom (*E*-4.40) or oxygen (*E*-4.31). Consequently the portion of the ring containing the corresponding ester bond is slightly different in each structure, due to the different bond lengths and angles of the ester of 4.31 compared to thio ester of *E*-4.40 (refer to Table 4.1 for ester bond lengths and angles).

Another difference between the two structures, as shown in Figure 4.17, is the position of the exocyclic methyl ester, where the C11-O4 carbonyl is almost at right angles in each case (refer to Table 4.1 for torsion angles of C10 – C9 – C11 – O4/O3). This is the result of the different cell constants in each crystal structure. Both crystalline compounds had an orthorhombic P2(1)2(1)2(1) space group, but serine-based *E*-4.31 had unit cell constants of 8.13, 9.05, and 16.93 Å, while cysteine-based *E*-4.40 had unit cell constants of 4.96, 15.60, and 16.71 Å. In other words the crystals of *E*-4.31 and *E*-4.40 were not isomorphous, i.e. despite their very similar chemical composition they did not have the same unit cell constants. The different packing in each crystal structure was a result of free rotation about the C9 – C11 bond, thus the exocyclic methyl ester adopted the most thermodynamically

favourable orientation in the unit cell of the crystal structures, which happened to be mutually at right angles in each case.

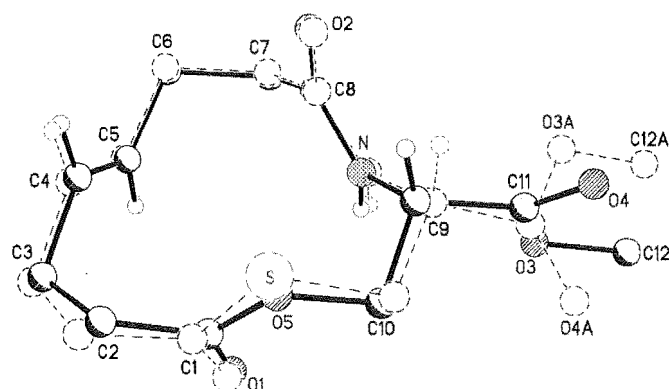


Figure 4.17. Superposition of crystal structure *E-4.31* (solid line) and *E-4.40* (dashed line).

Serine-based <i>E-4.31</i>		Cysteine-based <i>E-4.40</i>	
Bond length (Å)		Bond length (Å)	
C1 – O5	1.34 (16)	C1 – S	1.77 (2)
O5 – C10	1.42 (16)	S – C10	1.80 (18)
Bond angle		Bond angle	
C2 – C1 – O5	111.33 (11)	C2 – C1 – S	113.37 (14)
C1 – O5 – C10	117.40 (11)	C1 – S – C10	100.63 (9)
O5 – C10 – C9	109.02 (11)	S – C10 – C9	112.43 (13)
Torsion Angle		Torsion Angle	
C10 – C9 – C11 – O4	-92.58 (15)	C10 – C9 – C11 – O4	-0.3 (3)
C10 – C9 – C11 – O3	85.37 (13)	C10 – C9 – C11 – O3	-179.34 (16)

Table 4.1. Comparison of the x-ray crystal structures of *E-4.31* and *E-4.40*. Standard deviations given in brackets.

Molecular modeling

Molecular modeling of **4.40** and **4.31** was carried out to compare to the x-ray crystal structures shown in Figure 4.15. The *E*- and *Z*-configurations of both **4.40** and **4.31** were constructed *in silico* using Schrödinger's Maestro build function and minimized using the MMFFs forcefield *in vacuo*, which is reputed to be the best for comparison to x-ray crystal structures.^{21,22} A low energy ensemble was generated for each configuration, that was then used to calculate a Boltzmann weighted average energy (Figure 4.18).

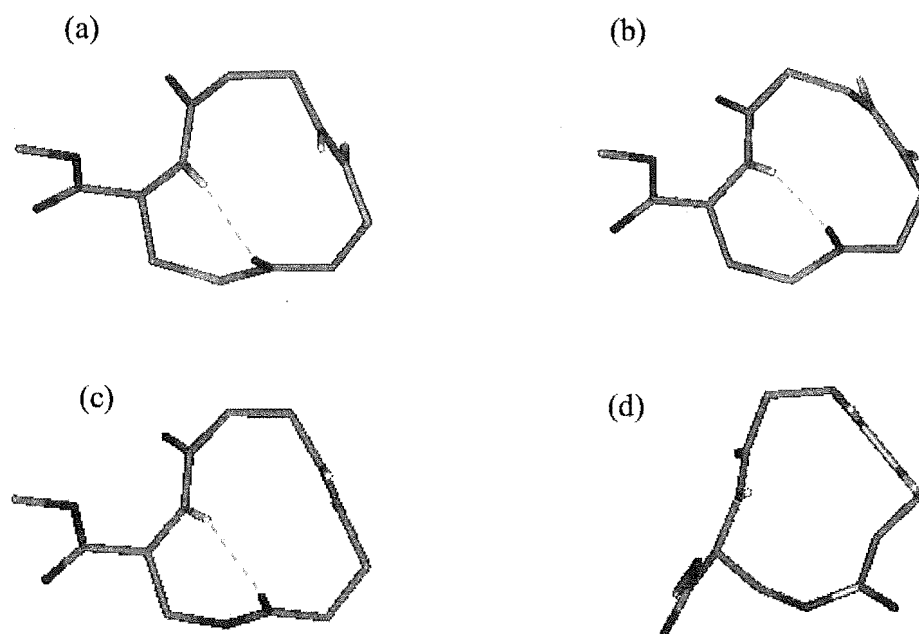


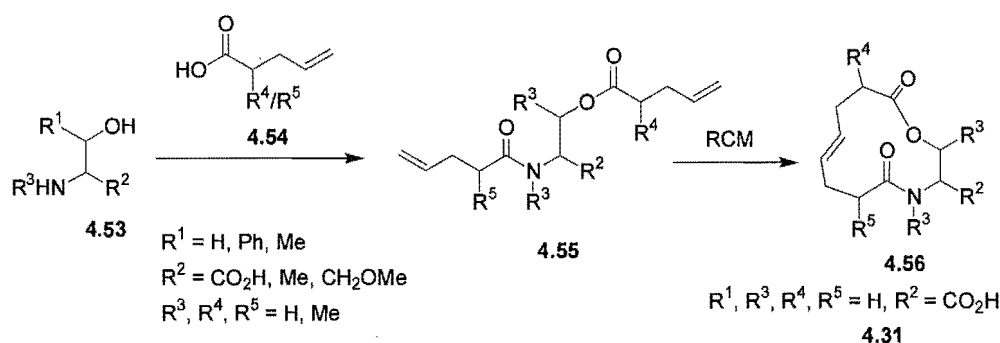
Figure 4.18. Minimized energy structures of (a) *E*-**4.40**, 12.81 kJ/mol; (b) *Z*-**4.40**, 16.99 kJ/mol; (c) *E*-**4.31**, 0.6 kJ/mol; (d) *Z*-**4.31**, 50.5 kJ/mol. Boltzmann weighted average energies given.

The *E* configuration of both **4.40** and **4.31** were shown by molecular modeling to be lower in energy than the corresponding *Z* configuration. This is in line with the *E* configuration of both the x-ray crystal structures of **4.40** and **4.31**, and confirms that the RCM reactions were under thermodynamic control. This result also validates the assignment of the configuration of other RCM products that was based on molecular modeling results. The lowest energy structures of *E*-**4.40** and *E*-**4.31** adopt the same hexagonal ring shape (see Figure 4.16) as observed for the crystals structures (Figure 4.15). The *Z* minimized

structures of **4.40** and **4.31** can not adopt the more favourable hexagonal shape as they contain a *cis* two-atom element, disrupting the stable pattern of alternating two-atom *trans* elements around the ring.

The minimized structures of *E*-**4.40** and *E*-**4.31** revealed an intramolecular hydrogen bond between the amide hydrogen and carbonyl oxygen of the pentenoyl ester. The crystal structures of *E*-**4.40** and *E*-**4.31** contained only an intermolecular hydrogen bond. These different types (*intra* vs. *inter*) of hydrogen bonds were a result of the molecular modeling conditions, where each structure was analysed as one isolated molecule, and as such could not contain any intermolecular interactions. The intramolecular hydrogen bond observed for *E*-**4.40** and *E*-**4.31** is presumably a reason for the slightly distorted hexagonal shape in comparison to the corresponding crystal structures.

The synthesis and x-ray crystal structure of 12-membered serine-base cyclic compound **4.31** has previously been reported by Schreiber and colleges (Scheme 4.14),¹⁵ in a study directed towards establishing the suitability of split-pool syntheses to RCM reactions.

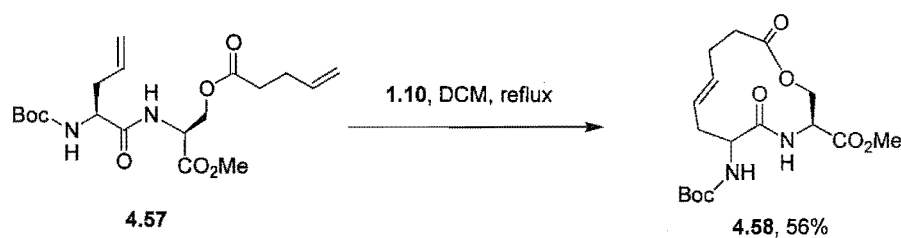


Scheme 4.14. Series of 12-membered compounds synthesis by Schreiber *et al.*¹⁵ Stereochemistry not shown.

The general methodology used to generate the series of structurally diverse cyclic compounds of the same ring size (**4.56**) shown in Scheme 4.14 involved using differently substituted starting materials **4.53** and **4.54**. The study presented in this thesis differs from that shown in Scheme 4.14 in that it focuses on the synthesis of compounds with varying ring sizes via controlled acylation of natural amino acid residues with variable lengths of carboxylic acids. The structure of compounds **4.56** were purposely chosen by Schreiber *et*

al as they contained the hexagonal-shaped 12-membered ring scaffold with low trans-annular and torsional strain, as discussed in Figure 4.16. Serine-based **4.31** was synthesised in this thesis, despite being a reported compound, to enable x-ray crystallography data to be collected at cryogenic 92 F, which were the running conditions of the in-house diffractometer. The Schreiber *et al* crystal structure of *E*-**4.31** was collected at 213 F, so was not suitable for direct comparison to the crystal structure of *E*-**4.40** obtained at 92 F in this thesis. Crystal structures obtained at these two temperatures would have significantly different unit cell constants.

A cyclic compound (**4.58**) similar in structure to *E*-**4.31** has also been reported in a patent by Grubbs and colleges²³ that covers the application of RCM for the construction of constrained peptides (Scheme 4.15). Serine-glycine-based RCM precursor **4.57** was cyclised using Grubbs first generation catalyst **1.10** to give 12-membered cyclic **4.58**. RCM product **4.58** has the same core ring structure as **4.31** but contained an additional exocyclic NHBoc group substituted in the ring scaffold, which is derived from the amino terminus of the allyl glycine residue in dipeptide-based **4.57**.



Scheme 4.15

4.4 CONCLUSION AND FUTURE WORK

The synthesis of 5 lysine- (**4.6**, **4.13**, **4.16**, **4.21**, **4.27**), 3 serine- (**4.31**, **4.33**, **4.37**), and 3 cysteine-based (**4.40**, **4.42**, **4.45**, **4.46**) cyclic amino acid and dipeptide compounds was carried out by RCM from the corresponding acyclic precursors. The 15-membered **4.6** and 27-membered **4.13** cyclic compounds were prepared by RCM of lysine-based acyclic precursors **4.5** and **4.12** respectively. These precursors were synthesised by side chain and *N*-terminus acylation of a single lysine residue with 4-pentenoic acid or 10-decenoic acid to install the different olefin tether lengths and therefore give cyclic compounds of different ring sizes. Lysine-based **4.6** was isolated as a mixture containing a ring-contracted product (**4.7a/b**), which was separated by semi-preparative HPLC and characterised.

Three lysine dipeptide-based cyclic compounds (**4.16**, **4.21**, **4.27**) were prepared via RCM of acyclic precursors (**4.15**, **4.20**, **4.26**) – **4.16** and **4.21** via a side-chain to side-chain cyclisation and **4.27** via a side-chain to *N*-terminus cyclisation. Acyclic dipeptide-based precursors **4.15**, **4.20**, **4.46** were synthesised using the same general methodology of acylation with carboxylic acids of controllable olefin tether lengths, providing the ability to easily produce an array of cyclic compounds with readily adjustable ring sizes.

Cyclic compounds containing a 12-membered (**4.31**) and 24-membered (**4.33**) ring, based on a single serine residue, were prepared via RCM of acyclic precursors **4.30** and **4.32**. A serine dipeptide-based cyclic compound, the 16-membered **4.37**, was prepared by RCM of a side-chain acylated serine dipeptide acyclic precursor (**4.36**). The analogous cyclic compounds based on a cysteine scaffold were also prepared (**4.40**, **4.42**, **4.45**).

The configuration of cyclic RCM products was assigned as the most thermodynamically stable isomer as shown by molecular modeling. Cysteine dipeptide-based cyclic *Z*-**4.45** and ring contracted RCM product **4.7a/b** were the only compounds assigned as the *Z* configuration. X-Ray crystal structures of **4.31** and **4.40** were obtained, and showed the same core ring structure. The observed *E* configuration in both x-ray crystal structures (**4.31**, **4.40**) was also the most thermodynamically stable configuration determined by molecular modeling, thus validating the modeling results.

The synthesis of dipeptide-based cyclic compound **4.46** demonstrates that the general methodology involving acylation of natural amino acid functional groups followed by RCM is a viable route to the core of PDF inhibitor **4.47**.

Future work could involve the application of this general methodology to the synthesis of other amino acid and dipeptide-based cyclic compounds that can be suitably acylated with a carboxylic acid, to give cyclic compounds with variable ring sizes. Target compounds could be prepared in this way, designed with a specific biological role in mind. In addition to application of this general methodology, the cyclic compounds prepared in this thesis could be used as cyclic amino acid building blocks, in that they could be incorporated into peptides to potentially alter the biological or physical properties of the parent peptide in an intended manner. They could be added to an in-house library of compounds and screened against specific biological targets.

4.5 REFERENCES FOR CHAPTER FOUR

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CHAPTER FIVE

EXPERIMENTAL

5.1 GENERAL METHODS AND EXPERIMENTAL PROCEDURES

Melting Points

All melting points were obtained on an Electrothermal apparatus and are uncorrected.

Nuclear Magnetic Resonance

Proton NMR spectra were obtained on a Varian Inova spectrometer, operating at 500 MHz. Carbon NMR were obtained on a Varian Inova Unity 300 spectrometer, operating at 75 MHz, with a delay (D_1) of 1 second. All spectra, unless specified, were obtained at 23°C. Chemical shifts are reported in parts per million (ppm) on the δ scale. Solvents used for NMR analysis (reference peaks listed) included: DMSO- d_6 ($\text{CHD}_2\text{SOCD}_3$ at δ_{H} 2.60 ppm, $(\text{CD}_3)_2\text{SO}$ at δ_{C} 39.6 ppm); CDCl_3 (CHCl_3 at δ_{H} 7.25 ppm, CDCl_3 at δ_{C} 77.0 ppm); CD_3OD (CHD_2OD at δ_{H} 3.30 ppm, CD_3OD at δ_{C} 49.3 ppm). Two-dimensional NMR experiments included COSY, HSQC, HMBC and CIGAR, and all were obtained on the Varian Inova spectrometer operating at 500 MHz.

Small Molecule Mass Spectrometry

Electron impact mass spectra were detected on a Kratos MS80 RFA mass spectrometer operating at 4000 V (accelerating potential) and 70 eV (ionization energy) and using a source temperature of 200 - 250°C. Electrospray ionization mass spectra were detected on a micromass LCT TOF mass spectrometer, with a probe voltage of 3200 V, temperature of 150°C and a source temperature of 80°C. Direct ionization used 10 μL of a 10 $\mu\text{g/mL}$ solution, using a carrier solvent of 50% acetonitrile/ H_2O at a flow rate of 20 $\mu\text{L/min}$. Ionisation was assisted by the addition of 0.5% formic acid.

High Pressure Liquid Chromatography

Analytical HPLC was performed on a Shimadzu LC-10AD VP liquid chromatograph coupled to a SIL-10A VP autoinjector, a CTO-10A VP column oven set to 40°C and a SPD-M10A VP photodiode array detector. The system was controlled by a Shimadzu CLASS-VP (Version 5.02) software. For reverse phase HPLC a Phenomenex Prodigy C18

5-ODS (3 μ , 250 \times 4.6 mm) column was used with a flow rate of 1 mL/min with a standard HPLC solvent gradient system that comprised of variable concentration of water (Milli-Q) containing 0.05% TFA and acetonitrile (HPLC grade). Isocratic systems consisted of a 20 min run with 20% acetonitrile in water (0.05% TFA). Preparative HPLC was performed on a Shimadzu LC-4A instrument equipped with a UV Spectrophotometric Detector SPD-2AS (wavelength λ = 206 nm). A Phenomenex Jupoter C18 Proteo 90A (10 μ , 250 \times 10 mm) was used with a flow rate of 16 mL/min. An isocratic solvent system of 20% acetonitrile in water (0.05 % TFA) was used.

X-ray Crystallography

All measurements were made with a Seimens CCD area detector using graphite monochromised Mo K α (λ = 0.71073 Å) radiation at 92 F. The data reduction was performed using SAINT.¹ Intensities were corrected for Lorentz and polarization effects and for absorption using SADABS. Space groups were determined from systematic absences and checked for higher symmetry. The structures were solved by direct methods² using SHELXS, and refined on F³ with all data using full-matrix least squares procedures with SHELXL-97. All non-hydrogen atoms were refined with anisotropic displacement parameters. All hydrogen atoms were fixed in idealised positions. Absolute structure determinations were based on the Flack parameter. In all cases, final Fourier syntheses showed no significant residual electron density in chemical sensible positions.

Molecular Modeling

E- And *Z*- configurations of cyclic compounds were constructed in silico using Schrödinger's Maestro build function and minimized using the MMFFs forcefield in vacuo, which is reputed to be best for comparing with crystal structures.^{4,5} The same forcefield was then used to perform a conformational search on each structure. Conformations within 12 kJ mol⁻¹ Ång⁻¹ of the lowest energy conformation were saved to generate a low energy ensemble for each structure. Each ensemble was then used to calculate the Boltzmann weighted average energy of each compound allowing the most thermodynamically stable double bond configuration of each compound to be identified. Molecular modeling was carried out by Blair Stewart, Department of Chemistry, University of Canterbury.

Microanalysis

Microanalysis was performed at the University of Otago Microanalytical Laboratory.

Reagents, solvents and laboratory methodology

Oven-dried glassware was used in all reactions carried out under an inert atmosphere (refers to either dry argon or dry nitrogen). All starting materials and reagents were obtained commercially unless otherwise stated. "Removal of the solvent (or volatiles) by evaporation under reduced pressure" was carried out by rotary evaporation (low vacuum pump) followed by application of high vacuum (oil pump) for a minimum of one hour. Analytical TLC was performed on plastic-backed Merck Keisegel KG60F₂₅₄ silica plates, and visualised using ultraviolet light or potassium permanganate dip. Column chromatography was performed using 230-400 mesh Merck Silica Gel 60 under an atmospheric pressure unless stated. Flash column chromatography refers to the use of a positive pressure of nitrogen.⁶ THF and diethyl ether were distilled from sodium benzophenone ketyl under an inert atmosphere immediately prior to use. DCM, acetonitrile, and TEA were distilled from calcium hydride under an inert atmosphere immediately prior to use. Ethyl acetate and petroleum ether were distilled from calcium hydride prior to their use in chromatography. Petroleum ether refers to the fraction collected between 50 - 70°C. HPLC grade DMF was purchased commercially and stored over 4 Å molecular sieves in an inert atmosphere. All other reagents and solvents were purified prior to use using literature procedures.⁷

General Procedure I: RCM

To a solution of alkene (1 equiv) in dry DCM (~0.06 M) was added Grubbs second generation catalyst **1.17** (0.2 equiv). The solution was stirred at reflux under a flow of inert gas for 6 h, and DMSO added (50 equiv relative to catalyst) and stirring continued at rt overnight. The solvent was removed under reduced pressure to give the crude product.

General Procedure II: Esterification of carboxylic acids using 2,2-dimethoxypropane

To a suspension of carboxylic acid (1 equiv) in 2,2-dimethoxypropane (~0.1 M) was added concentrated 12 M aqueous hydrochloric acid (~0.1 M) and the solution was stirred at reflux for 2 h and then overnight at rt. The solvent was removed by evaporation under reduced pressure and the residue dissolved in a minimal amount of methanol. See individual experiments for recrystallisation details.

General Procedure III: Hydrogenation of olefins

Olefin (1 equiv) was dissolved in methanol (typically 0.02 M) and 10% palladium on carbon was added (~ 20% w/w) and the solution stirred overnight under an atmosphere of hydrogen gas (hydrogen balloon). The solution was filtered through celite and the solvent removed by evaporation under reduced pressure.

Modified General Procedure III: Hydrogenation of CBZ group

The substrate (1 equiv) was dissolved in methanol (typically 0.02 M) and 10% palladium on carbon was added (~ 20% w/w) and the solution stirred overnight under an atmosphere of hydrogen (hydrogen balloon). The solution was filtered through celite and the solvent removed by evaporation under reduced pressure.

General Procedure IV: Acylation of amines using acid chlorides⁸

To a solution of amine (1 equiv, ~0.1 M) in 1 M sodium hydroxide/acetonitrile (1:1 v/v) was added dropwise the acid chloride (1.1 equiv). The solution was stirred for 2 h, and then quenched with 1 M aqueous hydrochloric acid to give a pH of 5-6 (Universal indicator paper). The mixture was extracted twice with ethyl acetate, dried (Na₂SO₄), and the solvent removed by evaporation under reduced pressure.

General Procedure V: HATU Peptide coupling⁹

To a solution of the amine (1 equiv) and carboxylic acid (1 equiv) in DMF was added DIPEA (6 equiv). HATU was added (1.2 equiv) and the reaction stirred overnight. The reaction was quenched with water and the resulting solution extracted twice with ethyl acetate. The organic layer was washed twice with aqueous saturated sodium bicarbonate, saturated ammonium chloride, and saturated brine, then dried (MgSO_4), and the solvent removed by evaporation under reduced pressure.

General Procedure VI: Boc-cleavage using TFA

To a stirred solution of the Boc-protected compound (1 equiv) in DCM (~0.2 M) was added TFA (typically 40 equiv). The solution was stirred overnight, and then the solvent removed by evaporation under reduced pressure. The residue was re-dissolved in methanol and the solvent removed by evaporation under reduced pressure three times to remove any residual TFA.

General Procedure VII: EDCI Peptide coupling¹⁰

To a stirred solution of the amine (1 equiv) and carboxylic acid (1.14 equiv) in DCM was added EDCI (1.3 equiv) and HOBt (1.5 equiv). DIPEA (1.14 equiv) was added and the reaction stirred overnight. The solution was diluted with DCM, and the organic layer washed twice with saturated brine. The combined aqueous layers were back extracted twice with DCM, and the combined organic layers were dried (MgSO_4), and the solvent removed by evaporation under reduced pressure.

General Procedure VIII: Hydrolysis of methyl ester with lithium hydroxide

To a stirred solution of methyl ester (1 equiv) in THF at 0°C was added dropwise a solution of lithium hydroxide in water (0.2 M aqueous LiOH, 2 equiv). The solution was stirred at 0°C for 40 min and then quenched by addition to a biphasic system of 0.2 M aqueous hydrochloric acid/ethyl acetate (1:1 v/v). The aqueous layer was extracted with ethyl acetate, the organic layers combined and dried (MgSO_4), and the solvent removed by evaporation under reduced pressure.

General Procedure IX: CM Dimer formation

To a solution of alkene (2 equiv) in dry DCM (~0.05 M) was added Grubbs second generation catalyst **1.17** (0.2 equiv). The solution was refluxed under a flow of inert gas for 6 h, and DMSO added (50 equiv relative to catalyst) and stirred at rt overnight. The solvent was removed by evaporation under reduced pressure to give the crude product.

General Procedure X: CM

To a solution of amino acid-based alkene substrate (1 equiv) and coupling partner alkene substrate (3 equiv) in dry DCM (~0.05 M) was added Grubbs second generation catalyst **1.17** (0.2 equiv). The solution was stirred at reflux under a flow of inert gas for 6 h, and DMSO added (50 equiv relative to catalyst) and stirring continued at rt overnight. The solvent was removed by evaporation under reduced pressure to give the crude product.

General Procedure XI: BOP-Cl Acylation of a thiol¹¹

TEA (2.4 equiv) was added dropwise to a stirred solution of acid (1.1 equiv) in dry DCM (~0.09 M) under an inert atmosphere. After stirring for 20 min, this solution was added dropwise to a solution of BOP-Cl (1.2 equiv) in dry DCM (~0.09 M) at 0°C. A solution of thiol (1 equiv) in dry DCM (~0.09 M) was then added dropwise at 0°C. After 15 min the reaction was allowed to warm to rt and stirring continued overnight. The solvent was removed by evaporation under reduced pressure and the residue dissolved in ethyl acetate. The solution was washed twice with water, and the combined organic layers were washed with aqueous saturated sodium bicarbonate, water, and saturated brine solution sequentially. The combined organic layers were dried (MgSO₄), and the solvent removed by evaporation under reduced pressure.

General Procedure XII: Acylation of an alcohol using EDCI/DMAP¹²

To a stirred solution of alcohol (1 equiv) and carboxylic acid (1.14 equiv) in DCM (~0.1 M) was added DMAP (0.01 equiv) and the solution cooled to 0°C. EDCI (1.09 equiv) was added, the solution was stirred at 0°C for 2 h and then allowed to warm to rt with stirring overnight. The solvent was removed by evaporation under reduced pressure and the residue dissolved in ethyl acetate and water (1:1 v/v). The organic layer was separated, washed with saturated aqueous bicarbonate and water, and the resulting organic layer was dried (MgSO₄), and the solvent removed by evaporation under reduced pressure.

General Procedure XIV: Dihydropyran protection

To a solution of alcohol (1 equiv) and dihydropyran (5 equiv) in DCM (~0.13 mM) was added *p*-toluene sulfonic acid monohydrate (10 equiv) at 0°C. The solution was stirred for 10 min at 0°C then at rt for 2 h. Ether was added and the solution was washed with saturated brine, saturated aqueous sodium bicarbonate, and water. The resulting organic layer was dried (MgSO₄), and the solvent removed by evaporation under reduced pressure.

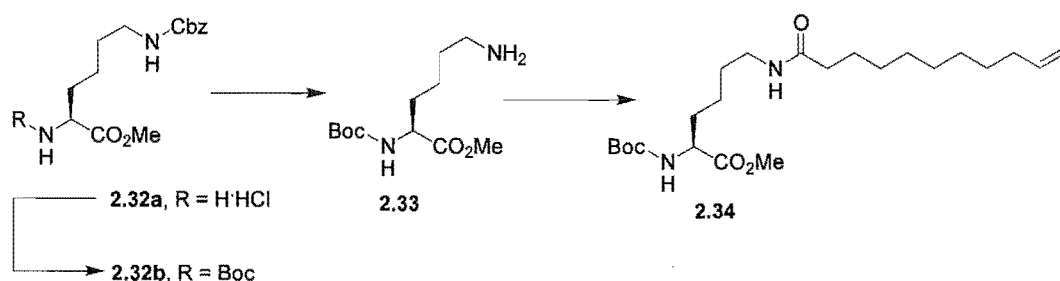
General Procedure XV: Esterification of carboxylic acid using Amberlyst 15 IER¹³

To a stirred solution of carboxylic acid in methanol (~0.2 mM) was added Amberlyst 15 Ion Exchange Resin (~170 mg/mmol carboxylic acid), and the suspension stirred at rt overnight. The Amberlyst IER was filtered off, and the resulting organic solvent removed by evaporation under reduced pressure.

5.2 EXPERIMENTAL WORK AS DESCRIBED IN CHAPTER TWO

5.2.1 Synthesis of Amino Acid Cross-Metathesis Precursors

Preparation of (2*S*)-2-*tert*-butoxycarbonylamino-6-(undec-10-enoylamino)-hexanoic acid methyl ester (2.34)



(2*S*)-2-*tert*-Butoxycarbonylamino-6-benzyloxycarbonylamino-hexanoic acid methyl ester (2.32b) To a stirred solution of Boc anhydride (1.32 g, 6.0 mmol, 1 equiv) and *N*_ε-Cbz-*L*-Lys-OMe·HCl **2.32a** (2 g, 6.0 mmol, 1 equiv) in acetonitrile (50 mL) was added TEA (2.6 mL, 18.0 mmol, 3 equiv). The solution was stirred for 16 h, DCM (50 mL) was added and the organic phase extracted with 1 M aqueous hydrochloric acid (50 mL) and saturated aqueous sodium bicarbonate. The organic layer was dried (MgSO₄), and the solvent removed by evaporation under reduced pressure to give **2.32b** (2.38 g) as a pale yellow oil which was not purified further.

¹H NMR (CDCl₃, 500 MHz) δ 1.23-1.60 (m, 4H, CH₂), 1.40 (s, 9H, C(CH₃)₃), 1.62 (br m, 1H, NHCHCHH), 1.75 (br m, 1H, NHCHCHH), 3.15 (m, 2H, NHCH₂), 3.69 (s, 3H, OCH₃), 4.24 (m, 1H, NHCH), 4.96 (br s, 1H, CHNH), 5.06 (s, 2H, OCH₂), 5.13 (br d, *J* = 7.9 Hz, 1H, CH₂NH), 7.26-7.32 (m, 5H, ArH).

6-Amino-2-*tert*-butoxycarbonylamino-hexenoic acid methyl ester (2.33) *N*_α-Boc-*N*_ε-Cbz-*L*-Lys-OMe **2.32b** (3.20 g, 8.10 mmol) was treated with 10% palladium on carbon (640 mg) under a hydrogen atmosphere according to Modified General Procedure III, to give a yellow oil that was not characterised and used without purification.

(2S)-2-tert-Butoxycarbonylamino-6-(undec-10-enoylamino)-hexanoic acid methyl ester (2.34) Boc-*L*-Lys-OMe.HCl **2.33** (500 mg, 1.68 mmol, 1 equiv) and 10-decenoic acid (353 mg, 1.92 mmol, 1.14 equiv) were treated with EDCI (417 mg, 2.18 mmol, 1.3 equiv) and HOBT (380 mg, 2.52 mmol, 1.5 equiv) according to General Procedure VII. The crude product was purified by flash chromatography on silica (petroleum ether/ethyl acetate, 3:7) to give **2.34** (418 mg, 58%) as a white solid.

mp = 63-65°C

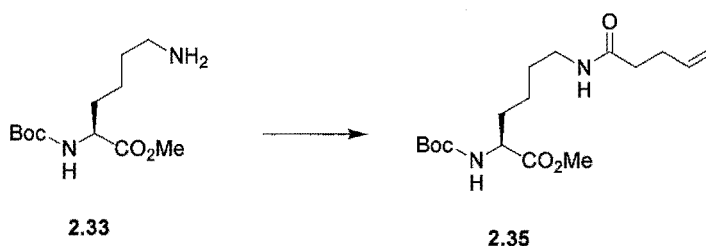
¹H NMR (CDCl₃, 500 MHz) δ 1.24-1.79 (m, 18H, CH₂), 1.42 (s, 9H, (CH₃)₃), 2.01 (m, 2H, (CO(CH₂)₇CH₂), 2.13 (t, *J* = 7.6 Hz, 2H, COCH₂), 3.21 (m, 2H, CH₂NH), 3.71 (s, 3H, OCH₃), 4.23 (m br, 1H, NHCH), 4.90 (m, 1H, CH=CHH), 4.96 (m, 1H, CH=CHH), 5.08 (d br, *J* = 7.8 Hz, 1H, NHCH), 5.56 (s br, 1H, CH₂NH), 5.78 (m, 1H, CH=CH₂).

¹³C NMR (CDCl₃, 75 MHz) δ 22.46, 25.67, 28.19, 28.74, 28.93, 29.18, 32.23, 33.64, 36.64, 38.84, 52.15, 53.04, 79.72, 114.02, 139.00, 155.43, 173.12, 173.23.

HRMS (M+Na) Found 449.2982 (Calcd for C₂₃H₄₂N₂O₅Na 449.2991).

Micro. Calcd for C₂₃H₄₂N₂O₅: C, 64.79; H, 9.86; N, 6.57. Found: C, 64.96; H, 10.07; N, 6.37.

(2S)-2-tert-Butoxycarbonylamino-6-(pent-4-enoylamino)-hexanoic acid methyl ester (2.35)



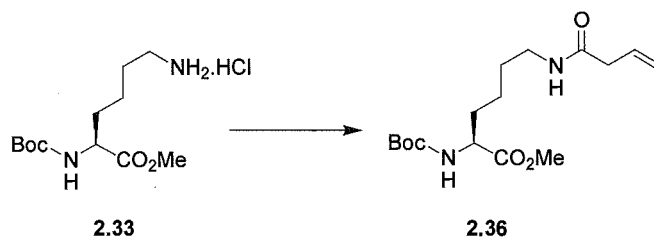
Boc-*L*-Lys-OMe.HCl **2.33** (2.11 g, 8.10 mmol, 1 equiv) and 4-pentenoic acid (1.18 g, 9.23 mmol, 1.14 equiv) were treated with EDCI (2.01 g, 10.5 mmol, 1.3 equiv) and HOBT (1.83 g, 12.2 mmol, 1.5 equiv) according to General Procedure VII. The crude product was purified by flash chromatography on silica (petroleum ether/ethyl acetate, 1:4) to give **2.35** (1.96 g, 71%) as a colourless oil.

^1H NMR (CDCl_3 , 500 MHz) δ 1.31-1.60 (m, 4H, CH_2CH_2), 1.39 (s, 9H, $(\text{CH}_3)_3$), 1.62 (m, 1H, NHCHCHH), 1.75 (m, 1H, NHCHCHH), 2.22 (m, 2H, COCH_2CH_2), 2.34 (m, 2H, COCH_2), 3.19 (m, 2H, CH_2NH), 3.69 (s, 3H, OCH_3), 4.22 (m br, 1H, NHCH), 4.95 (dd, $J = 1.3, 10.3$ Hz, 1H, $=\text{CHH}$), 5.01 (dd, $J = 1.3, 17.1$ Hz, 1H, $=\text{CHH}$), 5.14 (d br, $J = 7.5$ Hz, 1H, NHCH), 5.74-5.83 (m, 2H, $\text{CH}=\text{CH}_2$, CH_2NH).

^{13}C NMR (CDCl_3 , 75 MHz) δ 22.10, 27.70, 28.40, 29.15, 31.13, 34.92, 38.22, 51.53, 52.89, 78.95, 114.64, 136.59, 155.19, 172.37, 172.79.

HRMS ($\text{M}+\text{H}$) Found 343.2227 (Calcd for $\text{C}_{17}\text{H}_{31}\text{N}_2\text{O}_5$ 343.2233).

(2S)-2-tert-Butoxycarbonylamino-6-(but-3-enoylamino)-hexanoic acid methyl ester (2.36)

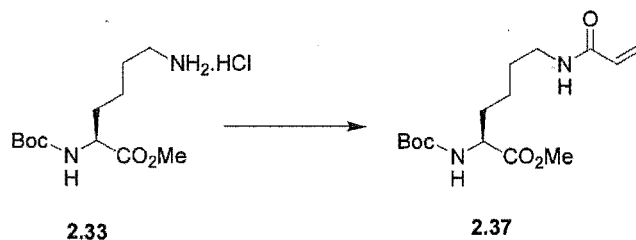


Boc-*L*-Lys-OMe.HCl **2.33** (50 mg, 0.17 mmol, 1 equiv) and vinyl acetic acid (13 mg, 0.15 mmol, 1.14 equiv) were treated with EDCI (42 mg, 0.22 mmol, 1.3 equiv) and HOBT (39 mg, 0.26 mmol, 1.5 equiv) according to General Procedure VII. The crude product was purified by flash chromatography on silica (petroleum ether/ethyl acetate, 1:4) to give **2.36** (41 mg, 77%) as a colourless oil.

^1H NMR (CDCl_3 , 500 MHz) δ 1.34 (m, 2H, CH_2), 1.42 (s, 9H, $\text{C}(\text{CH}_3)_3$), 1.50 (m, 2H, CH_2), 1.62 (m, 1H, NHCHCHH), 1.79 (m, 1H, NHCHCHH), 2.97 (d, $J = 7.2$ Hz, 2H, COCH_2), 3.22 (m, 2H, CH_2NH), 3.71 (s, 3H, OCH_3), 4.25 (m, 1H, CHNH), 5.07 (d br, $J = 8.0$ Hz, 1H, NHCH), 5.19 (m, 2H, $\text{CH}=\text{CH}_2$), 5.71 (s br, 1H, NHCH_2), 5.90 (m, 1H, $\text{CH}=\text{CH}_2$).

^{13}C NMR (CDCl_3 , 75 MHz) δ 22.48, 28.23, 28.9, 32.28, 39.09, 41.55, 52.22, 53.08, 79.84, 119.57, 131.42, 155.42, 170.59, 173.15.

HRMS Found ($\text{M} + \text{Na}$) 351.1901 (Calcd for $\text{C}_{16}\text{H}_{28}\text{N}_2\text{O}_5\text{Na}$ 351.1896).

(2S)-2-tert-Butoxycarbonylamino-6-acryloylamino-hexanoic acid methyl ester (2.37)

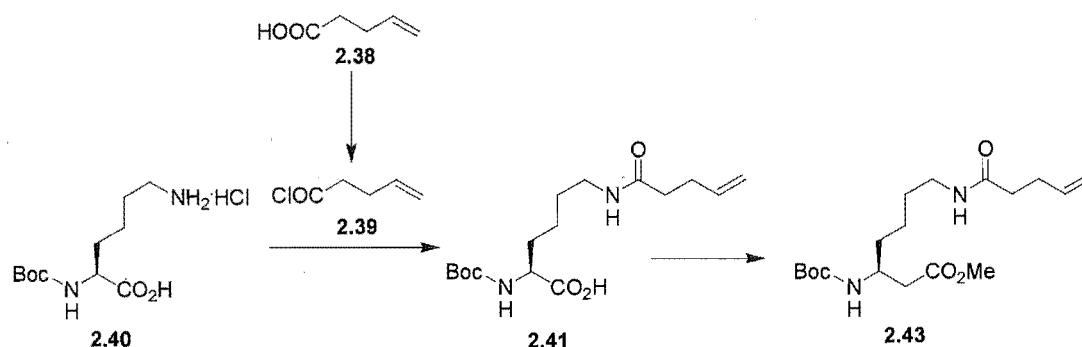
To a stirred solution of Boc-*L*-Lys-OMe.HCl **2.33** (1.5 g, 5.05 mmol, 1 equiv) in dry DCM (60 mL) under an inert atmosphere was added DIPEA at 0°C to give a pH of 8 to 9 (Universal indicator paper). Acryloyl chloride (842 mg, 9.30 mmol, 1.8 equiv) was added dropwise and the reaction was allowed to warm to rt with stirring overnight. The solution was washed successively with 1M aqueous hydrochloric acid (20 mL) and water (20 mL). The resulting organic layer was dried (MgSO₄), and the solvent removed by evaporation under reduced pressure. The crude product was purified by flash chromatography on silica (petroleum ether/ethyl acetate, 1:4) to give **2.37** (1.42 g, 78%) as a pale yellow, sticky gum.

¹H NMR (CDCl₃, 500 MHz) δ 1.32 (m, 2H, CH₂(CH₂)₂NH), 1.43 (s, 9H, (CH₃)₃), 1.54–1.69 (m, 3H, CH₂CH₂NH, NHCHCHH), 1.79 (m, 1H, NHCHCHH), 3.32 (q, *J* = 6.5 Hz, 2H, CH₂NH), 3.72 (s, 3H, OCH₃), 4.27 (m br, 1H, NHCH), 5.11 (d br, *J* = 7.8 Hz, 1H, NHCH), 5.61 (dd, *J* = 1.2, 10.3 Hz, 1H, CH=CHH), 5.78 (s br, 1H, CH₂NH), 6.08 (dd, *J* = 10.3, 17.0 Hz, 1H, COCH=), 6.27 (dd, *J* = 1.3, 17.0 Hz, 1H, CH=CHH).

¹³C NMR (CDCl₃, 75 MHz) δ 22.50, 28.21, 28.75, 32.22, 38.97, 52.22, 53.07, 79.83, 126.15, 130.81, 155.52, 165.72, 173.18.

HRMS Found (*M* + Na) 337.1740 (Calcd for C₁₅H₂₆N₂O₅Na 337.1738).

Preparation of (3*S*)-3-*tert*-butoxycarbonylamino-7-(pent-4-enoylamino)-heptanoic acid methyl ester (2.43)



4-Pentenoyl chloride (2.39) To a stirred solution of 4-pentenoic acid **2.38** (10 g, 0.1 mol, 1 equiv) in dry ether (120 mL) under an inert atmosphere was added dropwise oxalyl chloride (13.96 g, 0.11 mol, 1.1 equiv) at 0°C. After 10 min DMF (catalytic, a few drops), was added and the reaction was allowed to warm to rt and stirring continued overnight. The acid chloride product was purified via reduced pressure distillation to give 4-pentenoyl chloride **2.39** (9.65 g, 81%) as a colourless liquid.

(2*S*)-2-*tert*-Butoxycarbonylamino-6-(pent-4-enoylamino)-hexanoic acid (2.41) *N*-Boc-*L*-Lys-OH.HCl **2.40** (2 g, 8.12 mmol, 1 equiv) was treated with 4-pentenoyl chloride **2.39** (1.06 g, 8.93 mmol, 1.1 equiv) according to General Procedure IV. The crude product was recrystallised (petroleum ether/ethyl acetate) to give **2.41** (829 mg, 31%) as a wax.

¹H NMR (CDCl₃, 500 MHz) 1.38 (m, 2H, NHCHCH₂CH₂), 1.43 (s, 9H, C(CH₃)₃), 1.52 (m, 2H, NHCH₂CH₂), 1.70 (m, 1H, NHCHCHH), 1.84 (m, 1H, NHCHCHH), 2.28 (m, 2H, COCH₂CH₂), 2.36 (m, 2H, m, 2H, COCH₂), 3.23 (m, 2H, NHCH₂), 4.26 (m, 1H, NHCH), 4.99 (d, *J* = 10.1 Hz, 1H, CH=CHH), 5.05 (d, *J* = 17.2 Hz, 1H, CH=CHH), 5.30 (br d, *J* = 7.8 Hz, 1H, CHNH), 5.79 (m, 1H, CH=CH₂), 5.97 (br s, 1H, NHCH₂).

¹³C NMR (CDCl₃, 75 MHz) 28.11, 28.52, 29.51, 31.88, 35.34, 39.03, 53.01, 60.27, 79.66, 115.39, 136.62, 155.67, 173.52, 174.92.

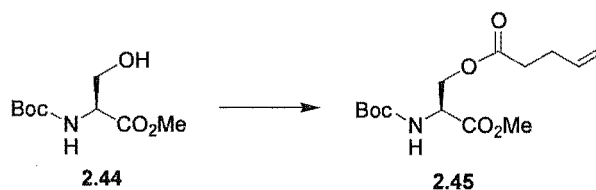
(3S)-3-tert-Butoxycarbonylamino-7-(pent-4-enoylamino)-heptanoic acid methyl ester (2.43) TEA (202 mg, 2 mmol, 1 equiv) and ethyl chloroformate (217 mg, 2 mmol, 1 equiv) were added under an inert atmosphere to N_{α} -Boc- N_{ϵ} -pentenoyl-L-Lys-OH **2.41** (655 mg, 2 mmol, 1 equiv) in THF (10 mL) at -15°C . After 15 min the suspension was allowed to warm to 0°C , whereupon an excess of diazomethane in ether ($\sim 0.2\text{ M}$) was added until the intensive yellow colour persisted over time. The mixture was allowed to warm to rt with stirring for 3 h. Excess diazomethane was destroyed by the addition of a few drops of acetic acid. The solution was washed with aqueous saturated sodium bicarbonate (10 mL), saturated ammonium chloride (10 mL), and saturated brine solution (10 mL), the organic layer dried (MgSO_4), and the solvent removed by evaporation under reduced pressure. The crude diazoketone was dissolved in dry methanol (8 mL) under an inert atmosphere at -25°C with the exclusion of light. Silver benzoate (51 mg, 0.22 mmol, 0.11 equiv), dissolved in TEA (5.8 mmol, 2.9 equiv) was added and the reaction was allowed to warm to rt with stirring for 3 h. The solvent was removed by evaporation under reduced pressure and the residue was taken up in ethyl acetate. The organic layer was washed with aqueous saturated sodium bicarbonate (10 mL), saturated ammonium chloride (10 mL), and saturated brine solution (10 mL), the organic layer dried (MgSO_4), and the solvent was removed by evaporation under reduced pressure. The crude product was purified twice by flash chromatography on silica (petroleum ether/ethyl acetate, 1:9, then methanol/DCM 7.5:92.5) to give **2.43** (521 mg, 73%) as a pale yellow oil.

^1H NMR (CDCl_3 , 500 MHz) δ 1.31 (s, 9H, $\text{C}(\text{CH}_3)_3$), 1.33 (m, 6H, $\text{CH}(\text{CH}_2)_3$), 2.15 (t, $J = 7.4\text{ Hz}$, 2H, NHCOCH_2), 2.26 (m, 2H, $=\text{CHCH}_2$), 2.39 (d, $J = 5.7\text{ Hz}$, 2H, CHCH_2CO), 3.11 (m, 2H, CH_2NH), 3.56 (s, 3H, OCH_3), 3.77 (m, 1H, NHCH), 4.87 (dd, $J = 1.0, 10.2\text{ Hz}$, 1H, $\text{CH}=\text{CHH}$), 4.93 (dd, $J = 1.4, 17.1\text{ Hz}$, 1H, $\text{CH}=\text{CHH}$), 5.07 (br d, $J = 8.9\text{ Hz}$, 1H, NHCH), 5.70 (m, 1H, $\text{CH}=\text{CH}_2$), 6.21 (br s, 1H, CH_2NH).

^{13}C NMR (CDCl_3 , 75 MHz) δ 22.93, 28.13, 28.83, 29.45, 33.86, 35.5, 38.76, 39.21, 47.13, 51.41, 78.94, 115.10, 136.92, 155.36, 171.81, 172.36.

HRMS ($\text{M}+\text{H}$) Found 357.2389 (Calcd for $\text{C}_{18}\text{H}_{33}\text{N}_2\text{O}_5$ 357.2389).

(2*S*)-Pent-4-enoic acid-(2-*tert*-butoxycarbonylamino-2-methoxycarbonyl)-ethyl ester (2.45)



Boc-*L*-Ser-OMe **2.44** (2 g, 9.10 mmol, 1 equiv) and 4-pentenoic acid (1.04 g, 10.4 mmol, 1.14 equiv) were treated with EDCI (1.89g, 9.92 mmol, 1.09 equiv) and DMAP (11 mg, 0.09 mmol, 0.01 equiv) according the General Procedure XII. The crude product was purified by flash chromatography on silica (ethyl acetate/petroleum ether, 3:7) to give **2.45** (2.51 g, 89%) as a white solid.

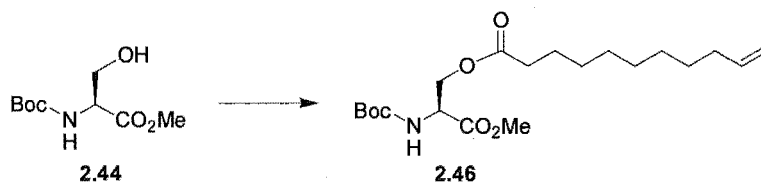
mp = 35-37°C

^1H NMR (CDCl_3 , 500 MHz) δ 1.42 (s, 9H, $\text{C}(\text{CH}_3)_3$), 2.32 (m, 2H, COCH_2CH_2), 2.39 (m, 2H, COCH_2), 3.73 (s, 3H, OCH_3), 4.31 (dd, $J = 3.6, 11.3$ Hz, 1H, OCHH), 4.41 (dd, $J = 3.6, 11.3$ Hz, 1H, OCHH), 4.54 (m, 1H, NHCH), 4.98 (dd, 1H, $J = 1.4, 10.3$ Hz, CH=CHH), 5.02 (dd, 1H, $J = 1.4, 17.2$ Hz, CH=CHH), 5.27 (br d, $J = 8.0$ Hz, 1H, NH), 5.77 (m, 1H, CH=CH_2).

^{13}C NMR (CDCl_3 , 75 MHz) δ 27.67, 28.13, 32.59, 51.98, 52.38, 63.46, 79.34, 115.02, 136.00, 154.67, 169.74, 171.79.

HRMS ($\text{M}+\text{H}$) Found 302.1601 (Calcd for $\text{C}_{14}\text{H}_{24}\text{NO}_6$ 302.1604).

(2*S*)-Undec-10-enoic acid-(2-*tert*-butoxycarbonylamino-2-methoxycarbonyl)-ethyl ester (2.46)



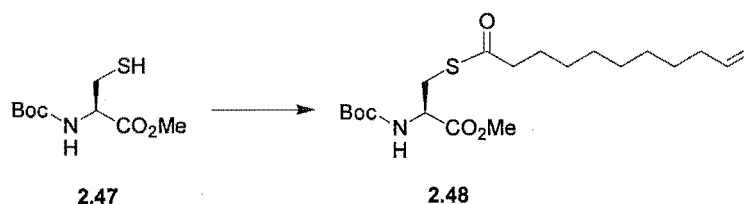
Boc-*L*-Ser-OMe **2.44** (300 mg, 1.37 mmol, 1 equiv) and 10-decenoic acid (287 mg, 1.56 mmol, 1.14 equiv) were treated with EDCI (285 mg, 1.49 mmol, 1.09 equiv) and DMAP (2 mg, 0.01 mmol, 0.01 equiv) according the General Procedure XII. The crude product was purified by flash chromatography on silica (ethyl acetate/petroleum ether, 1:4) to give **2.46** (444 mg, 84%) as a colourless oil.

^1H NMR (CDCl_3 , 500 MHz) δ 1.26-1.45 (m, 10H, CH_2), 1.43 (s, 9H, $(\text{CH}_3)_3$), 1.57 (m, 2H, COCH_2CH_2), 2.01 (m, 2H, $\text{CO}(\text{CH}_2)_7\text{CH}_2$), 2.27 (t, $J = 7.6$ Hz, 2H, COCH_2), 3.74 (s, 3H, OCH_3), 4.29 (dd, 1H, $J = 3.5, 11.2$ Hz, OCHH), 4.43 (dd, 1H, $J = 3.5, 11.2$ Hz, OCHH), 4.54 (m, 1H, NHCH), 4.90 (m, 1H, CH=CHH), 4.96 (m, 1H, CH=CHH), 5.27 (br d, $J = 8.0$ Hz, 1H, NH), 5.78 (m, 1H, CH=CHH).

^{13}C NMR (CDCl_3 , 75 MHz) δ 24.47, 27.94, 28.54, 28.71, 28.86, 28.95, 33.45, 33.60, 52.27, 52.66, 63.63, 79.74, 113.89, 138.67, 154.85, 169.99, 172.85.

HRMS ($\text{M}+\text{Na}$) Found 408.2361 (Calcd for $\text{C}_{20}\text{H}_{35}\text{NO}_6\text{Na}$ 408.2362).

(2*R*)-2-*tert*-Butoxycarbonylamino-3-(undec-10-enoylsulfanyl)-propionic acid methyl ester (2.48)



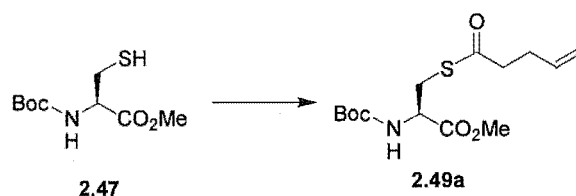
Boc-*L*-Cys-OMe **2.47** (598 mg, 2.54 mmol, 1 equiv) and 10-decenoic acid (516 mg, 2.80 mmol, 1.1 equiv) were treated with BOP-Cl (774 mg, 3.05 mmol, 1.2 equiv) according to General Procedure XI. The crude product was purified by flash chromatography on silica (ethyl acetate/petroleum ether, 1:4) to give **2.48** (840 mg, 82%) as a colourless oil.

^1H NMR (CDCl_3 , 500 MHz) δ 1.26-1.47 (m, 10H, CH_2), 1.42 (s, 9H, $(\text{CH}_3)_3$), 1.63 (m, 2H, COCH_2CH_2), 2.01 (m, 2H, $\text{CO}(\text{CH}_2)_7\text{CH}_2$), 2.53 (t, $J = 7.6$ Hz, 2H, COCH_2), 3.32 (m, 2H, CH_2S), 3.73 (s, 3H, OCH_3), 4.51 (m br, 1H, NHCH), 4.91 (m, 1H, $=\text{CHH}$), 4.97 (m, 1H, $=\text{CHH}$), 5.22 (d br, $J = 6.7$ Hz, 1H, NH), 5.79 (m, 1H, CH=CH_2).

^{13}C NMR (CDCl_3 , 75 MHz) δ 24.97, 27.67, 28.25, 28.29, 28.46, 28.62, 28.66, 30.25, 33.19, 43.28, 51.85, 52.56, 79.10, 113.70, 138.28, 154.49, 170.35, 197.44.

HRMS ($\text{M}+\text{Na}$) Found 424.2119 (Calcd for $\text{C}_{20}\text{H}_{35}\text{NO}_5\text{SNa}$ 424.2134).

(2*R*)-2-*tert*-Butoxycarbonylamino-3-(pent-4-enoylsulfanyl)-propionic acid methyl ester (2.49a)



Boc-*L*-Cys-OMe **2.47** (572 mg, 2.43 mmol, 1 equiv) and 4-pentenoic acid (267 mg, 2.67 mmol, 1.1 equiv) were treated with BOP-Cl (741 mg, 2.92 mmol, 1.2 equiv) according to General Procedure XI. The crude product was purified by flash chromatography on silica (ethyl acetate/petroleum ether, 1:4) to give **2.49a** (717 mg, 93%) as a white solid.

mp = 33–35°C

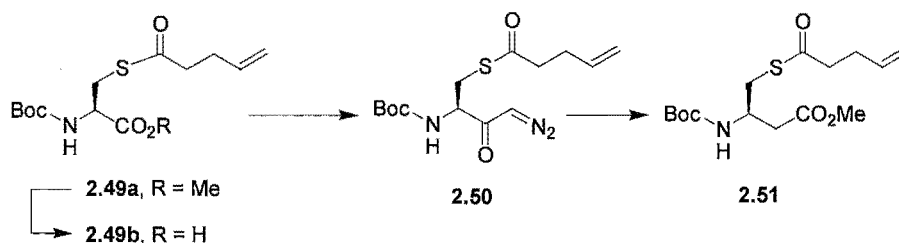
^1H NMR (CDCl_3 , 500 MHz) δ 1.42 (s, 9H, $(\text{CH}_3)_3$), 2.38 (m, 2H, COCH_2CH_2), 2.64 (t, J = 7.5 Hz, 2H, COCH_2), 3.33 (m, 2H, CH_2S), 3.73 (s, 3H, OCH_3), 4.51 (m br, 1H, NHCH), 5.00 (dd, J = 1.3, 10.3 Hz, 1H, $=\text{CHH}$), 5.04 (m, 1H, $=\text{CHH}$), 5.21 (d br, J = 7.5 Hz, 1H, NH), 5.76 (m, 1H, $\text{CH}=\text{CH}_2$).

^{13}C NMR (CDCl_3 , 75 MHz) δ 27.54, 28.63, 30.10, 42.25, 51.79, 52.42, 79.01, 115.25, 135.34, 154.43, 170.25, 196.64.

HRMS ($\text{M}+\text{H}$) Found 318.1375 (Calcd for $\text{C}_{14}\text{H}_{24}\text{NO}_5\text{S}$ 318.1375).

Micro. Calcd for $\text{C}_{14}\text{H}_{23}\text{NO}_5\text{S}$: C, 53.00; H, 7.26; N, 4.42; S, 10.09. Found: C, 52.77; H, 7.47; N, 4.41; S, 9.95.

(3*R*)-3-*tert*-Butoxycarbonylamino-4-(pent-4-enoylsulfanyl)-butyric acid methyl ester (2.51)



N-Boc-S-pentenoyl-*L*-Cys-OMe **2.49a** (440 mg, 1.45 mmol, 1 equiv) was treated with 0.2 M aqueous lithium hydroxide (2 equiv) according to General Procedure VIII to give **2.49b** which was not characterised. The carboxylic acid product was dissolved in THF (10 mL), and TEA (153 mg, 1.52 mmol, 1.05 equiv) and ethyl chloroformate (165 mg, 1.52 mmol,

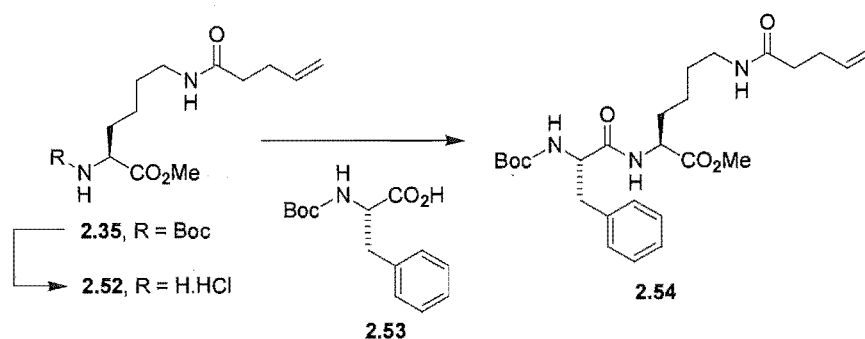
1.05 equiv) were added under an inert atmosphere at -15°C . After 15 min the suspension was allowed to warm to 0°C , whereupon diazomethane in ether ($\sim 0.2\text{ M}$) was added until the intensive yellow colour persisted over time. The mixture was allowed to warm to rt with stirring for 3 h. Excess diazomethane was destroyed by the addition of a few drops of acetic acid. The solution was washed with aqueous saturated sodium bicarbonate (10 mL), saturated ammonium chloride (10 mL), and saturated brine solution (10 mL), the organic layer dried (MgSO_4), and the solvent was removed by evaporation under reduced pressure. The crude product was purified by flash chromatography on silica (ethyl acetate/DCM, 1:19) to give pure diazoketone **2.50** (390 mg, 82%) as a yellow oil. Diazoketone **2.50** (390 mg, 1.19 mmol, 1 equiv) was dissolved in dry methanol (5 mL) under an inert atmosphere at -25°C with the exclusion of light. Silver benzoate (30 mg, 0.13 mmol, 0.11 equiv), dissolved in TEA (349 mg, 3.46 mmol, 2.9 equiv), was added and the reaction was allowed to warm to rt over 3 hrs. The solvent was removed by evaporation under reduced pressure and the residue was taken up in ethyl acetate. The organic layer was washed with aqueous saturated sodium bicarbonate (10 mL), saturated ammonium chloride (10 mL), and saturated brine solution (10 mL), the organic layer dried (MgSO_4), and the solvent was removed by evaporation under reduced pressure. The crude product was purified by flash chromatography on silica (ethyl acetate/petroleum ether, 1:4) to give **2.51** (170 mg, 51%) as a colourless oil.

^1H NMR (CDCl_3 , 500 MHz) δ 1.41 (s, 9H, $\text{C}(\text{CH}_3)_3$), 2.39 (m, 2H, COCH_2CH_2), 2.55 (m, 2H, COCH_2CH_2), 2.65 (m, 2H, $\text{CH}_2\text{CO}_2\text{Me}$), 3.13 (m, 2H, SCH_2), 3.68 (s, 3H, OCH_3), 4.06 (m, 1H, NHCH), 5.00 (dd, $J = 1.2, 10.2\text{ Hz}$, 1H, $\text{CH}=\text{CHH}$) 5.05 (dd, $J = 1.2, 17.1\text{ Hz}$, 1H, $\text{CH}=\text{CHH}$) 5.10 (br d, $J = 7.5\text{ Hz}$, 1H, NH) 5.77 (m, 1H, $\text{CH}=\text{CH}_2$).

^{13}C NMR (CDCl_3 , 75 MHz) δ 27.94, 29.03, 32.03, 37.36, 42.68, 47.30, 51.36, 78.99, 115.57, 135.63, 154.73, 171.03, 197.78.

HRMS ($\text{M}+\text{H}$) Found 332.1530 (Calcd for $\text{C}_{15}\text{H}_{26}\text{NO}_5\text{S}$ 332.1532).

(2*S*, 2*S*)-2-(2-*tert*-Butoxycarbonylamino-3-phenyl-propionylamino)-6-(pent-4-enoylamino)-hexanoic acid methyl ester (2.54**)**



*N*_α-Boc-*N*_ε-pentenoyl-*L*-Lys-OMe **2.35** (86 mg, 0.25 mmol) was dissolved in ethyl acetate and hydrochloric gas was bubbled through the solution for 4 h to give a solid suspension. The solid was filtered, washed with ether, and dried under high vacuum. The resulting residue (**2.52**, 0.25 mmol) and Boc-*L*-Phe-OH **2.53** (76 mg, 0.29 mmol, 1.14 equiv) were treated with EDCI (62 mg, 0.33 mmol, 1.3 equiv) and HOBT (57 mg, 0.38 mmol, 1.5 equiv) according to General Procedure VII. The crude product was purified by flash chromatography on silica (petroleum ether/ethyl acetate, 1:9) to give **2.54** (78 mg, 63%) as a white solid.

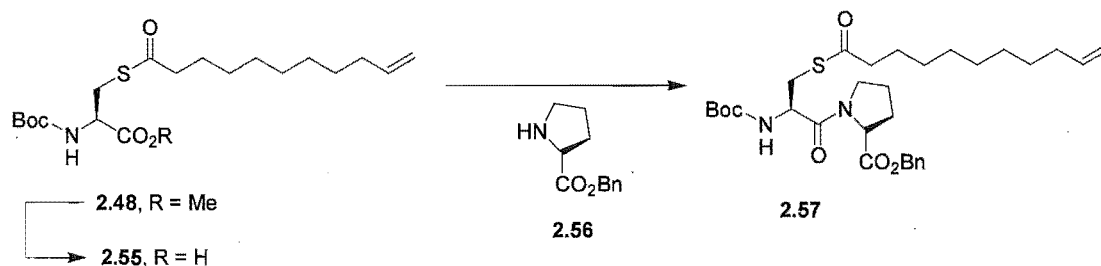
mp = 81-82°C

¹H NMR (CDCl₃, 500 MHz) δ 1.26-1.50 (m, 4H, CH₂), 1.38 (s, 9H, (CH₃)₃), 1.64 (m, 1H, NHCH_{Lys}CHH), 1.78 (m, 1H, NHCH_{Lys}CHH), 2.27 (m, 2H, COCH₂CH₂), 2.36 (m, 2H, COCH₂), 3.00-3.23 (m, 4H, CH₂NH, CH₂Ph), 3.69 (s, 3H, OCH₃), 4.36 (m br, 1H, NHCH_{Phe}), 4.51 (m br, 1H, NHCH_{Lys}), 4.98 (dd, *J* = 1.8, 10.3 Hz, 1H, =CHH), 5.03 (ddd, *J* = 1.8, 3.5, 17.1 Hz, 1H, =CHH), 5.13 (s br, 1H, NHCH_{Phe}), 5.78 (m, 1H, CH=CH₂), 5.95 (s br, 1H, NHCH₂), 6.66 (d br, *J* = 7.3 Hz, 1H, NHCH_{Lys}), 7.18-7.45 (m, 5H, ArH).

¹³C NMR (CDCl₃, 75 MHz) δ 22.24, 28.16, 28.62, 29.63, 31.61, 35.65, 38.22, 38.86, 52.02, 52.33, 55.70, 80.20, 115.51, 126.83, 128.51, 129.23, 136.44, 136.87, 155.51, 171.69, 172.15, 173.25.

HRMS (M+Na) Found 512.2734 (Calcd for C₂₆H₃₉N₃O₆Na 512.2737).

(2*R*, 2*S*)-1-[2-*tert*-Butoxycarbonylamino-3-(undec-10-enoylsulfanyl)-propionyl]-pyrrolidine-2-carboxylic acid benzyl ester (2.57)



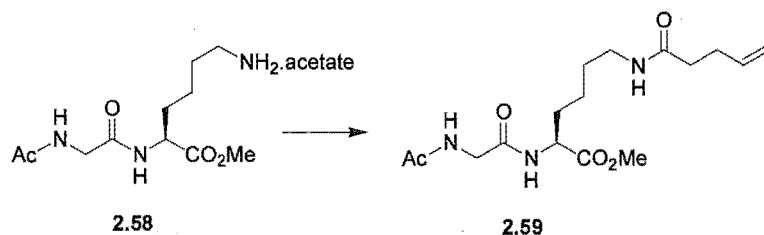
Cysteine derivative **2.48** (400 mg, 1.0 mmol) was hydrolysed by treatment with 0.2 M aqueous lithium hydroxide (2 equiv) according to General Procedure VIII to give **2.55**, which was not characterised. Carboxylic acid **2.55** (1.0 mmol, 1 equiv) and *L*-Pro-OBn.HCl **2.56** (275 mg, 1.13 mmol, 1.14 equiv) were treated with EDCI (248 mg, 1.3 mmol, 1.3 equiv) and HOBt (227 mg, 1.5 mmol, 1.5 equiv) according to General Procedure VII. The crude product was purified by flash chromatography on silica (ethyl acetate/DCM, 1:9) to give **2.57** (414 mg, 72%) as a yellow oil.

^1H NMR (CDCl_3 , 500 MHz) δ 1.38 (s, 9H, $\text{C}(\text{CH}_3)_3$), 1.62 (m, 2H, COCH_2CH_2), 1.99 (m, 4H, NCH_2CH_2 , $=\text{CHCH}_2$), 2.17 (m, 2H, CHCH_2 _{proline}), 2.52 (t, $J = 7.5$ Hz, 2H, COCH_2), 2.70 (m, 1H, SCHH), 3.30 (m, 1H, SCHH), 3.71 (m, 1H, NCHH), 3.91 (m, 1H, NCHH), 4.55 (m, 2H, NHCH , NCH), 4.89 (dd, $J = 0.8, 10.2$ Hz, 1H, $\text{CH}=\text{CHH}$), 4.95 (m, 1H, $\text{CH}=\text{CHH}$), 5.07 (d, $J = 12.3$ Hz, 1H, OCHH), 5.17 (d, $J = 12.3$ Hz, 1H, OCHH), 5.26 (m, 1H, NH), 5.77 (m, 1H, $\text{CH}=\text{CH}_2$), 7.30 (m, 5H, ArH).

^{13}C NMR (CDCl_3 , 75 MHz) δ 24.46, 25.09, 27.90, 28.44, 28.55, 28.62, 28.66, 28.77, 28.80, 31.28, 33.35, 43.53, 46.59, 51.04, 58.58, 66.38, 79.14, 113.82, 127.70, 127.83, 128.11, 135.22, 138.58, 155.07, 168.97, 170.97, 198.82.

HRMS ($\text{M}+\text{Na}$) Found 597.2964 (Calcd for $\text{C}_{31}\text{H}_{46}\text{N}_2\text{O}_6\text{NaS}$ 597.2974).

(2*S*)-2-(2-Acetylamino-acetylamino)-6-(pent-4-enoylamino)-hexanoic acid methyl ester (2.59)



Dipeptide acetate salt **2.58** (50 mg, 0.16 mmol, 1 equiv) and 4-pentenoic acid (18 mg, 0.18 mmol, 1.14 equiv) were treated with EDCI (40 mg, 0.21 mmol, 1.3 equiv) and HOBT (36 mg, 0.24 mmol, 1.5 equiv) according to General Procedure VII. The crude product was purified by flash chromatography on silica (methanol/DCM, 1:9) to give **2.59** (50 mg, 93%) as a white solid.

mp = 115-116°C

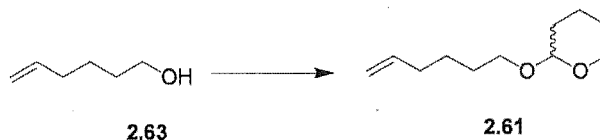
^1H NMR (CDCl_3 , 500 MHz) δ 1.32 (m, 2H, CHCH_2CH_2), 1.49 (m, 2H, $\text{CH}_2\text{CH}_2\text{NH}$), 1.79 (m, 2H, NHCHCH_2), 2.05 (s, 3H, COCH_3), 2.28 (m, 2H, COCH_2CH_2), 2.38 (m, 2H, COCH_2CH_2), 3.44 (m, 2H, CH_2NH), 3.73 (s, 3H, OCH_3), 3.96 (m, 2H, NHCH_2CO), 4.54 (m, 1H, NHCH), 5.00 (d, 1H, $J = 10.3$ Hz, $\text{HHC}=\text{CH}$), 5.06 (d, 1H, $J = 17.1$ Hz, $\text{HHC}=\text{CH}$), 5.82 (m, 2H, $\text{H}_2\text{C}=\text{CH}$, $\text{CH}_2\text{CH}_2\text{NH}$), 6.40 (br s, 1H, NHCH_2CO), 6.74 (d, 1H, $J = 7.80$ Hz, NHCH)

^{13}C NMR (CDCl_3 , 75 MHz) δ 22.18, 22.92, 28.89, 29.64, 31.20, 35.75, 38.52, 43.15, 52.06, 52.42, 115.50, 137.04, 169.67, 170.87, 172.49, 172.78.

HRMS ($\text{M}+\text{H}$) Found 342.2036 (Calcd for $\text{C}_{16}\text{H}_{28}\text{N}_3\text{O}_5$ 342.2029).

5.2.2 Synthesis of protected alcohol terminal alkene CM products

2-Hex-5-enyloxy-tetrahydro-pyran (2.61)

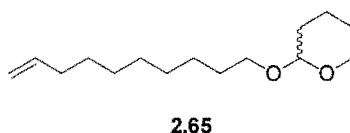


5-Hexen-1-ol **2.63** (400 mg, 4.00 mmol, 1 equiv) and dihydropyran (2.04 g, 20.0 mmol, 5 equiv) were treated with *p*-toluene sulfonic acid monohydrate (7.60 g, 40.0 mmol, 10 equiv) according to General Procedure XIV. The crude product was purified by chromatography on silica (ethyl acetate/petroleum ether, 1:9) to give **2.61** (734 mg, 99%) as a colourless liquid.

^1H NMR (CDCl_3 , 500 MHz) δ 1.42-1.68 (m, 8H, CH_2), 1.70 (m, 1H, OCHCHH), 1.80 (m, 1H, OCHCHH), 2.05 (m, 2H, $=\text{CHCH}_2\text{CH}_2$), 3.36 (m, 1H, OCHH), 3.47 (m, 1H, OCHH), 3.71 (m, 1H, OCHH), 3.83 (m, 1H, OCHH), 4.54 (m, 1H, OCHO), 4.91 (m, 1H, HHC=CH), 4.98 (ddd, $J = 2.0, 3.9, 17.6$ Hz, 1H, HHC=CH), 5.78 (m, 1H, $\text{CH}_2=\text{CH}$).

^{13}C NMR (CDCl_3 , 75 MHz) δ 19.31, 25.28, 25.39, 28.95, 30.47, 33.32, 61.78, 67.01, 98.39, 114.18, 138.37.

2-Dec-9-enyloxy-tetrahydro-pyran (2.65)

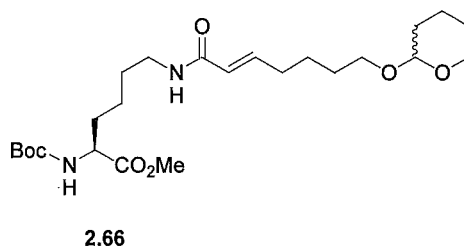


9-Decen-1-ol **2.64** (876 mg, 5.60 mmol, 1 equiv) and dihydropyran (2.86 g, 28.0 mmol, 5 equiv) were treated with *p*-toluene sulfonic acid monohydrate (10.7 g, 56.0 mmol, 10 equiv) according to General Procedure XIV. The crude product was purified by chromatography on silica (ethyl acetate/petroleum ether, 3:17) to give **2.65** (1.21 g, 90%) as a colourless liquid.

^1H NMR (CDCl_3 , 500 MHz) δ 1.24-1.61 (m, 16H, CH_2), 1.69 (m, 1H, OCHCHH), 1.81 (m, 1H, OCHCHH), 2.01 (q, $J = 7.1$ Hz, 2H, $=\text{CHCH}_2\text{CH}_2$), 3.36 (m, 1H, OCHH), 3.48 (m, 1H, OCHH), 3.71 (m, 1H, OCHH), 3.85 (m, 1H, OCHH), 4.55 (m, 1H, OCHO), 4.90 (m, 1H, $\text{HHC}=\text{CH}$), 4.97 (ddd, $J = 2.0, 3.4, 17.1$ Hz, 1H, $\text{HHC}=\text{CH}$), 5.79 (m, 1H, $\text{CH}_2=\text{CH}$).

^{13}C NMR (CDCl_3 , 75 MHz) δ 19.59, 25.43, 26.15, 28.82, 28.99, 29.34, 29.66, 30.69, 33.71, 62.17, 67.55, 98.70, 114.02, 139.04.

(2*S*)-2-*tert*-Butoxycarbonylamino-6-[7-(tetrahydro-pyran-2-yloxy)-hept-2-enoylamino]-hexanoic acid methyl ester (2.66)



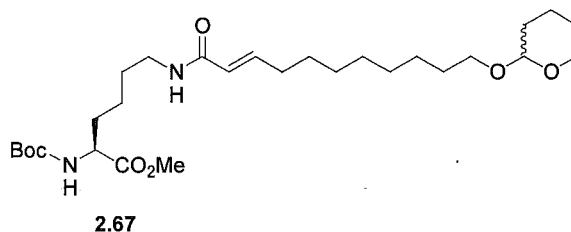
*N*_ε-Acryloyl lysine **2.37** (20 mg, 0.64 mmol, 1 equiv) was reacted with **2.61** (35 mg, 0.20 mmol, 3 equiv) and Grubbs second generation catalyst **1.17** (11 mg, 0.01 mmol, 0.2 equiv) according to General Procedure X. The crude product was purified by chromatography on silica (petroleum ether/ethyl acetate, 1:4) to give **2.66** (27 mg, 91%) as a brown oil.

^1H NMR (CDCl_3 , 500 MHz) δ 1.32–1.80 (m, 16H, CH_2), 1.39 (s, 9H, $(\text{CH}_3)_3$), 2.15 (m, 2H, $=\text{CHCH}_2$), 3.25 (m, 2H, CH_2NH), 3.33 (m, 1H, OCHH), 3.45 (m, 1H, OCHH), 3.69 (s, 3H, OCH_3), 3.73 (m, 1H, OCHH), 3.80 (m, 1H, OCHH), 4.21 (m br, 1H, NHCH), 4.52 (m, 1H, OCHO), 5.15 (d br, $J = 7.93$ Hz, 1H, CHNH), 5.74 (d, $J = 15.1$ Hz, 1H, COCH=), 5.85 (s br, 1H, CH_2NH), 6.77 (m, 1H, $=\text{CHCH}_2$).

^{13}C NMR (CDCl_3 , 75 MHz) δ 19.63, 22.56, 24.99, 25.45, 28.31, 28.95, 29.25, 30.72, 31.79, 32.51, 39.05, 52.31, 53.06, 62.33, 67.16, 79.92, 98.85, 123.69, 144.39, 155.57, 166.06, 173.24.

HRMS ($\text{M}+\text{H}$) Found 471.3071 (Calcd for $\text{C}_{24}\text{H}_{43}\text{N}_2\text{O}_7$ 471.3070).

(2*S*)-2-*tert*-Butoxycarbonylamino-6-[11-(tetrahydro-pyran-2-yloxy)-undec-2-enoyl-amino]-hexanoic acid methyl ester (2.67)



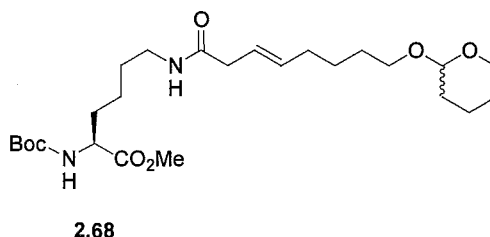
*N*_ε-Acryloyl lysine **2.37** (20 mg, 0.06 mmol, 1 equiv) was reacted with **2.65** (47 mg, 0.20 mmol, 3 equiv) and Grubbs second generation catalyst **1.17** (11 mg, 0.01 mmol, 0.2 equiv) according to General Procedure X. The crude product was purified by chromatography on silica (petroleum ether/ethyl acetate, 2:3) to give **2.67** (25 mg, 75%) as a brown oil.

¹H NMR (CDCl₃, 500 MHz) δ 1.21–1.79 (m, 24H, CH₂), 1.39 (s, 9H, C(CH₃)₃), 2.10 (m, 2H, CH=CHCH₂), 3.25 (m, 2H, CH₂NH), 3.33 (m, 1H, OCHHCH₂), 3.45 (m, 1H, OCHHCH₂), 3.67 (s, 3H, OCH₃), 3.68 (m, 1H, OCHHCH₂), 3.82 (m, 1H, OCHHCH₂), 4.22 (m, 1H, NHCH), 4.52 (m, 1H, OCHO), 5.15 (d, *J* = 8.3 Hz, 1H, CHNH), 5.73 (d, *J* = 15.1 Hz, 1H, COCH=), 5.86 (br s, 1H, CH₂NH), 6.77 (m, 1H, COCH=CH).

¹³C NMR (CDCl₃, 75 MHz) δ 19.60, 22.50, 25.37, 26.07, 28.12, 28.21, 28.85, 29.00, 29.22, 29.23, 29.59, 30.67, 31.89, 32.28, 38.91, 52.17, 53.03, 62.26, 67.54, 79.76, 98.77, 123.40, 144.62, 155.47, 166.17, 173.15.

HRMS (M+H) Found 527.3700 (Calcd for C₂₈H₅₁N₂O₇ 527.3696).

(2*S*)-2-*tert*-Butoxycarbonylamino-6-[8-(tetrahydro-pyran-2-yloxy)-oct-3-enoylamino]-hexanoic acid methyl ester (2.68)



*N*_ε-Propenoyl lysine **2.36** (20 mg, 0.58 mmol, 1 equiv) was reacted with **2.61** (32 mg, 0.18 mmol, 3 equiv) and Grubbs second generation catalyst **1.17** (11 mg, 0.01 mmol, 0.2 equiv)

according to General Procedure X. The crude product was purified by chromatography on silica (petroleum ether/ethyl acetate, 1:4) to give a mixture (16 mg, 57%) of **2.68** and the double bond migrated product **2.68a** as a brown oil (3:1 by ^1H NMR).

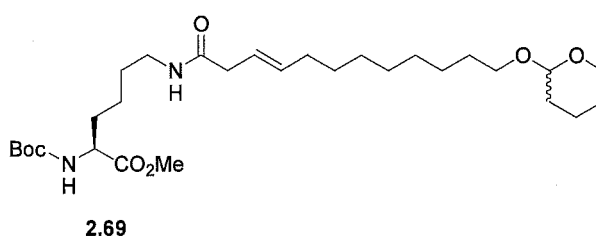
^1H NMR (CDCl_3 , 500 MHz) from mixture **2.68** δ 1.22–1.81 (m, 14H, CH_2), 1.41 (s, 9H, $\text{C}(\text{CH}_3)_3$), 2.06 (m, 2H, $\text{CH}=\text{CHCH}_2\text{CH}_2$), 2.89 (d, $J = 6.8$ Hz, 2H, COCH_2), 3.19 (m, 2H, CH_2NH), 3.35 (m, 1H, OCHHCH_2), 3.45 (m, 1H, OCHHCH_2), 3.70 (s, 3H, OCH_3), 3.71 (m, 1H, OCHHCH_2), 3.84 (m, 1H, OCHHCH_2), 4.24 (m, 1H, NHCHCO), 4.53 (m, 1H, OCHO), 5.08 (d, $J = 7.8$ Hz, 1H CHNH), 5.48 (dt, $J = 7.0, 15.3$ Hz, 1H, $\text{CH}=\text{CH}$), 5.58 (dt, $J = 6.7, 15.3$ Hz, 1H, $\text{CH}=\text{CH}$), 5.76 (m, 1H, CH_2NH).

Selected data ^1H NMR (CDCl_3 , 500 MHz) from mixture **2.68a** δ 2.17 (m, $\text{CH}=\text{CHCH}_2\text{CH}_2$), 2.96 (d, $J = 7.8$ Hz, $\text{COCH}_2\text{CH}=\text{}$), 3.27 (m, CH_2NH), 5.19 (br m, CHNH), 5.64 (m, $\text{CH}=\text{CH}$), 5.79 (m, CH_2NH).

^{13}C NMR (CDCl_3 , 75 MHz) from mixture **2.68** δ 19.63, 22.50, 25.37, 25.76, 28.22, 28.97, 29.14, 30.67, 32.25, 35.11, 39.06, 40.42, 52.19, 53.08, 62.32, 67.27, 79.78, 98.83, 122.84, 135.89, 155.39, 171.27, 173.27.

HRMS ($\text{M}+\text{H}$) Found 485.3224 (Calcd for $\text{C}_{25}\text{H}_{45}\text{N}_2\text{O}_7$ 485.3227).

(2*S*)-2-*tert*-Butoxycarbonylamino-6-[12-(tetrahydro-pyran-2-yloxy)-dodec-3-enoyl-amino]-hexanoic acid methyl ester (2.69)



N_ϵ -Propenoyl lysine **2.36** (20 mg, 0.06 mmol, 1 equiv) was reacted with **2.65** (42 mg, 0.18 mmol, 3 equiv) and Grubbs second generation catalyst **1.17** (10 mg, 0.01 mmol, 0.2 equiv) according to General Procedure X. The crude product was purified by chromatography on silica (petroleum ether/ethyl acetate, 1:4) to give a mixture (18 mg, 56%) of **2.69** and the double migrated product **2.69a** as brown oil (3:1 by ^1H NMR).

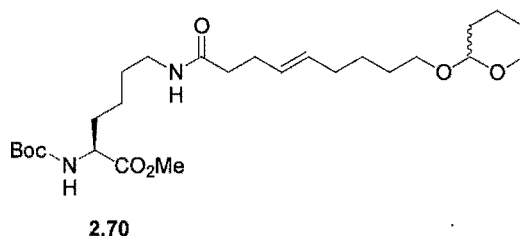
^1H NMR (CDCl_3 , 500 MHz) from mixture **2.69** δ 1.24-1.71 (m, 22H, CH_2), 1.41 (s, 9H, $\text{C}(\text{CH}_3)_3$), 1.78 (m, 2H, $=\text{CHCH}_2\text{CH}_2$), 2.01 (m, 2H, $=\text{CHCH}_2\text{CH}_2$), 2.89 (d, $J = 7.0$ Hz, 2H, COCH_2), 3.19 (m, 2H, CH_2NH), 3.34 (m, 1H, OCHH), 3.46 (m, 1H, OCHH), 3.69 (m, 1H, OCHH), 3.70 (s, 3H, OCH_3), 3.83 (m, 1H, OCHH), 4.24 (br m, 1H, CHNH), 4.54 (m, 1H, OCHO), 5.08 (br d, $J = 8.0$ Hz, 1H, NHCH), 5.47 (dt, $J = 7.1, 15.4$ Hz, 1H, $=\text{CH}$), 5.57 (dt, $J = 6.7, 15.3$ Hz, 1H, $=\text{CH}$), 5.72 (br s, 1H, CH_2NH).

Selected data ^1H NMR (CDCl_3 , 500 MHz) from mixture **2.69a** δ 2.96 (d, $J = 7.6$ Hz, $\text{COCH}_2\text{CH}=\text{}$), 3.27 (m, CH_2NH), 5.13 (br m, CHNH), 5.64 (m, $\text{CH}=\text{CH}$), 5.74 (m, CH_2NH).

^{13}C NMR (CDCl_3 , 75 MHz) from mixture **2.69** δ 19.62, 22.49, 25.39, 26.12, 28.22, 28.98, 29.04, 29.07, 29.29, 29.32, 29.63, 30.68, 32.24, 32.46, 39.06, 40.43, 52.19, 53.08, 62.28, 67.57, 79.79, 98.77, 122.46, 136.36, 155.38, 171.36, 173.13.

HRMS ($\text{M}+\text{H}$) Found 541.3854 (Calcd for $\text{C}_{29}\text{H}_{53}\text{N}_2\text{O}_7$ 541.3853).

(2*S*)-2-*tert*-Butoxycarbonylamino-6-[9-(tetrahydro-pyran-2-yloxy)-non-4-enoyl-amino]-hexanoic acid methyl ester (2.70**)**



N_ϵ -Pentenoyl lysine **2.35** (9 mg, 0.03 mmol, 1 equiv) was reacted with **2.61** (15 mg, 0.08 mmol, 3 equiv) and Grubbs second generation catalyst **1.17** (4 mg, 0.01 mmol, 0.2 equiv) according to General Procedure X. The crude product was purified by chromatography on silica (petroleum ether/ethyl acetate, 1:4) to give a mixture (7 mg, 54%) of **2.70** and the double bond migrated product **2.70a** as a brown oil (6:1 by ^1H NMR).

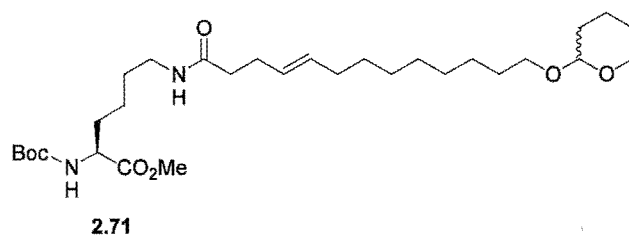
^1H NMR (CDCl_3 , 500 MHz) from mixture **2.70** δ 1.33-1.81 (m, 16H, CH_2), 1.42 (s, 9H, $\text{C}(\text{CH}_3)_3$), 1.99 (m, 2H, $=\text{CHCH}_2(\text{CH}_2)_3$), 2.19 (m, 2H, COCH_2), 2.29 (m, 2H, COCH_2CH_2), 3.21 (m, 2H, CH_2NH), 3.36 (m, 1H, OCHH), 3.47 (m, 1H, OCHH), 3.70 (m, 1H, OCHH), 3.71 (s, 3H, OCH_3), 3.83 (m, 1H, OCHH), 4.25 (br m, 1H, CHNH), 4.54 (m, 1H, OCHO), 5.10 (br d, $J = 7.2$ Hz, 1H, NHCH), 5.41 (dtd, $J = 6.6, 15.4, 21.9$ Hz, 2H, $\text{CH}=\text{CH}$), 5.63 (br s, 1H, CH_2NH).

Selected data ^1H NMR (CDCl_3 , 500 MHz) from mixture **2.70a** δ 2.90 (d, $J = 6.8$ Hz, $\text{COCH}_2\text{CH=}$), 3.28 (m, CH_2NH), 5.49 (m, CH=CH).

^{13}C NMR (CDCl_3 , 75 MHz) from mixture **2.70** δ 19.67, 22.53, 25.45, 26.00, 28.29, 28.63, 29.00, 29.15, 30.74, 32.27, 32.38, 36.64, 38.97, 52.28, 53.10, 62.35, 67.43, 79.89, 98.85, 128.63, 131.48, 155.49, 172.62, 173.21.

HRMS ($\text{M}+\text{H}$) Found 499.3385 (Calcd for $\text{C}_{26}\text{H}_{47}\text{N}_2\text{O}_7$ 499.3383).

(2*S*)-2-*tert*-Butoxycarbonylamino-6-[13-(tetrahydro-pyran-2-yloxy)-tridec-4-enoyl-amino]-hexanoic acid methyl ester (2.71)



*N*_ε-Pentenoyl lysine **2.35** (50 mg, 0.15 mmol, 1 equiv) was reacted with **2.65** (105 mg, 0.44 mmol, 3 equiv) and Grubbs second generation catalyst **1.17** (25 mg, 0.03 mmol, 0.2 equiv) according to General Procedure X. The crude product was purified by chromatography on silica (petroleum ether/ethyl acetate, 1:4) to give a mixture (41 mg, 51%) of **2.71** and the double bond migrated products **2.71a** and **2.71b** as a brown oil (23:4:1 by ^1H NMR).

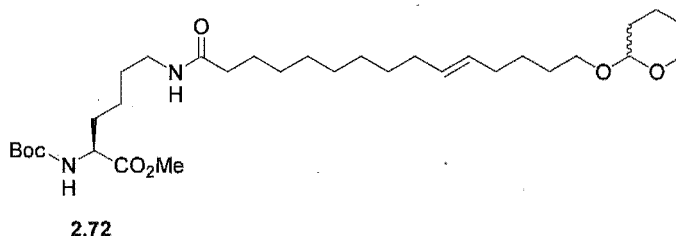
^1H NMR (CDCl_3 , 500 MHz) from mixture **2.71** δ 1.26–2.05 (m, 26H, CH_2), 1.42 (s, 9H, $(\text{CH}_3)_3$), 2.19 (m, 2H, COCH_2CH_2), 2.29 (m, 2H, COCH_2), 3.21 (m, 2H, CH_2NH), 3.36 (m, 1H, OCHH), 3.48 (m, 1H, OCHH), 3.71 (m, 1H, OCHH), 3.72 (s, 3H, OCH_3), 3.85 (m, 1H, OCHH), 4.26 (m br, 1H, NHCH), 4.55 (m, 1H, OCHO), 5.08 (br m, 1H, CHNH), 5.36 (dt, $J = 6.4, 15.4$ Hz, 1H, CH=CH), 5.45 (dt, $J = 6.6, 15.4$ Hz, 1H, CH=CH), 5.59 (m, 1H, CH_2NH).

Selected NMR data ^1H NMR (CDCl_3 , 500 MHz) from mixture **2.71a** and **b** δ 2.90 (d, $J = 7.0$ Hz, $\text{COCH}_2\text{CH=}$), 2.98 (d, $J = 7.6$ Hz, $\text{COCH}_2\text{CH=}$), 3.29 (m, CH_2NH), 5.49 (m, CH=CH), 5.66 (m, CH_2NH).

^{13}C NMR (CDCl_3 , 75 MHz) from mixture **2.71** δ 19.51, 22.39, 25.29, 26.01, 28.12, 28.50, 28.86, 28.92, 28.96, 29.03, 29.22, 29.52, 30.58, 32.05, 32.30, 36.42, 38.71, 52.06, 53.01, 62.152, 67.46, 79.615, 98.66, 128.14, 131.55, 155.38, 172.55, 173.05.

HRMS (M+H) Found 555.3998 (Calcd for C₃₀H₅₅N₂O₇ 555.4009).

(2*S*)-2-*tert*-Butoxycarbonylamino-6-[15-(tetrahydro-pyran-2-yloxy)-pentadec-10-enoylamino]-hexanoic acid methyl ester (2.72)



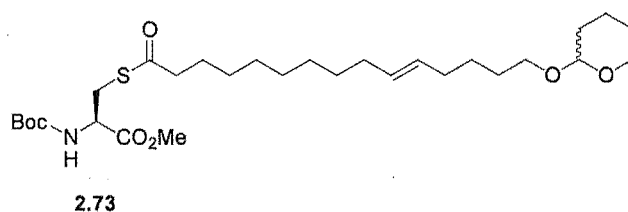
*N*_ε-Decenoyl lysine **2.34** (20 mg, 0.05 mmol, 1 equiv) was reacted with **2.61** (26 mg, 0.14 mmol, 3 equiv) and Grubbs second generation catalyst **1.17** (8 mg, 0.01 mmol, 0.2 equiv) according to General Procedure X. The crude product was purified by chromatography on silica (petroleum ether/ethyl acetate, 3:7) to give **2.72** (20 mg, 67%) as a brown oil.

¹H NMR (CDCl₃, 500 MHz) δ 1.26-1.82 (m, 28H, CH₂), 1.43 (s, 9H, C(CH₃)₃), 1.97 (m, 4H, CH₂CH=CH), 2.13 (t, *J* = 7.8 Hz, 2H, COCH₂), 3.22 (m, 2H, CH₂NH), 3.36 (m, 1H, OCHH), 3.48 (m, 1H, OCHH), 3.71 (m, 1H, OCHH), 3.72 (s, 3H, OCH₃), 3.85 (m, 1H, OCHH), 4.27 (m, 1H, NHCH), 4.56 (m, 1H, OCHO), 5.07 (br d, *J* = 8.3 Hz, 1H, NHCH), 5.34 (m, 2H, CH=CH), 5.54 (br s, 1H, CH₂NH).

¹³C NMR (CDCl₃, 75 MHz) δ 19.58, 22.47, 25.38, 25.68, 26.01, 27.06, 28.19, 28.93, 28.97, 29.00, 29.22, 29.32, 29.48, 29.62, 30.66, 32.27, 32.46, 36.68, 38.85, 52.16, 53.04, 62.21, 67.56, 79.74, 98.72, 130.15, 130.22, 155.42, 173.12, 173.17.

HRMS (M+H) Found 639.4929 (Calcd for C₃₆H₆₇N₂O₇ 639.4948).

(2*R*)-2-*tert*-Butoxycarbonylamino-3-[15-(tetrahydro-pyran-2-yloxy)-pentadec-10-enoylsulfanyl]-propionic acid methyl ester (2.73)



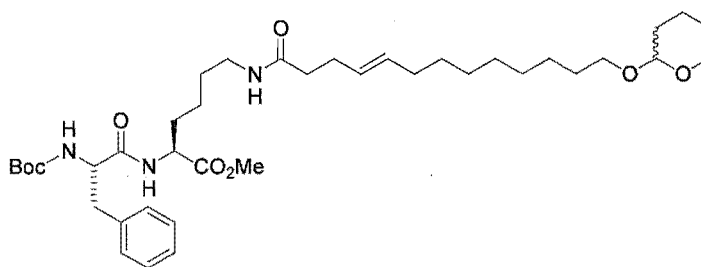
*N*_ε-Decenoyl cysteine **2.48** (100 mg, 0.25 mmol, 1 equiv) was reacted with **2.61** (138 mg, 0.75 mmol, 3 equiv) and Grubbs second generation catalyst **1.17** (42 mg, 0.05 mmol, 0.2 equiv) according to General Procedure X. The crude product was purified by chromatography on silica (petroleum ether/ethyl acetate, 1:4) to give **2.73** (50 mg, 36%) as a brown oil.

¹H NMR (CDCl₃, 500 MHz) δ 1.25-1.82 (m, 22H, CH₂), 1.42 (s, 9H, C(CH₃)₃), 1.99 (m, 4H, =CHCH₂), 2.53 (t, *J* = 7.5 Hz, 2H, COCH₂), 3.31 (m, 3H, OCHH, CH₂S), 3.47 (m, 1H, OCHH), 3.70 (m, 1H, OCHH), 3.73 (s, 3H, OCH₃), 3.84 (m, 1H, OCHH), 4.49 (m, 1H, CHNH), 4.56 (m, 1H, OCHO), 5.33 (m, 3H, NH, CH=CH).

¹³C NMR (CDCl₃, 75 MHz) δ 19.45, 25.32, 25.40, 26.05, 28.10, 28.67, 28.88, 29.03, 29.18, 29.35, 29.44, 30.56, 30.72, 32.19, 32.37, 43.79, 52.43, 52.93, 62.03, 67.28, 79.90, 98.56, 129.80, 130.44, 154.91, 170.83, 198.29.

HRMS (M+Na) Found 580.3289 (Calcd for C₂₉H₅₁NO₇SNa 580.3284).

(2*S*, 2*S*)-2-(2-*tert*-Butoxycarbonylamino-3-phenyl-propionylamino)-6-[13-(tetrahydro-pyran-2-yloxy)-tridec-4-enoylamino]-hexanoic acid methyl ester (2.74)



2.74

N_e-Pentenoyl dipeptide **2.54** (15 mg, 0.03 mmol, 1 equiv) was reacted with **2.65** (23 mg, 0.09 mmol, 3 equiv) and Grubbs second generation catalyst **1.17** (5 mg, 0.01 mmol, 0.2 equiv) according to General Procedure X. The crude product was purified by chromatography on silica (petroleum ether/ethyl acetate, 1:4) to give a mixture (12 mg, 56%) of **2.74** and double bond migrated product **2.74a** as a brown oil (5:1 by ¹H NMR).

¹H NMR (CDCl₃, 500 MHz) from mixture **2.74** δ 1.26-1.94 (m, 24H, CH₂), 1.39 (s, 9H, C(CH₃)₃), 1.83 (m, 2H, =CHCH₂(CH₂)₇), 2.21 (m, 2H, COCH₂), 2.30 (m, 2H, COCH₂CH₂), 3.07 (m, 2H, CH₂Ph), 3.20 (m, 2H, CH₂NH), 3.37 (m, 1H, OCHH), 3.48 (m, 1H, OCHH), 3.69 (s, 3H, OCH₃), 3.71 (m, 1H, OCHH), 3.86 (m, 1H, OCHH), 4.35 (m, 1H, NHCH_{Ph}), 4.51 (m, 1H, NHCH_{Lys}), 4.56 (m, 1H, OCHO), 5.10 (br m, 1H, CHNH_{Ph}), 5.37 (dt, *J* = 6.4, 15.3 Hz, 1H, CH=CH), 5.45 (dt, *J* = 6.5, 15.3 Hz, 1H, CH=CH), 5.71 (br m, 1H, CH₂NH), 6.56 (br d, *J* = 7.2 Hz, 1H, CHNH_{Lys}), 7.24 (m, 5H, ArH).

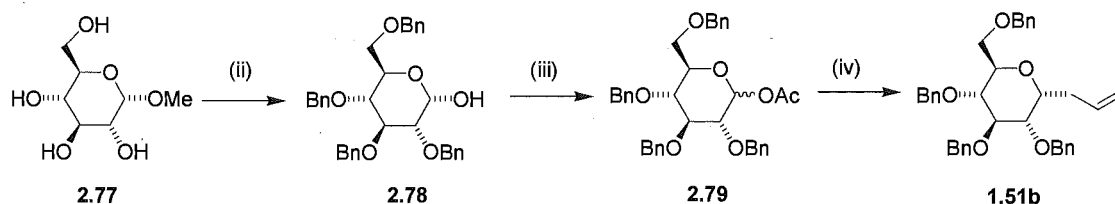
Selected data ¹H NMR (CDCl₃, 500 MHz) from mixture **2.74a** δ 2.92 (d, *J* = 7.0 Hz, COCH₂CH).

¹³C NMR (CDCl₃, 75 MHz) from mixture **2.74** δ 19.68, 22.10, 25.44, 26.17, 28.91, 29.11, 28.19, 28.66, 29.04, 29.39, 29.69, 30.74, 31.68, 32.46, 38.19, 36.62, 38.62, 51.91, 52.32, 55.71, 62.34, 67.64, 80.11, 98.83, 126.85, 128.23, 128.54, 129.29, 131.80, 136.54, 155.34, 171.24, 172.16, 172.81.

HRMS (M+H) Found 702.4692 (Calcd for C₃₉H₆₄N₃O₈ 702.4693).

5.2.3 Synthesis of Sugar CM compounds

Preparation of (2*R*, 3*R*, 4*S*, 5*R*, 6*R*)-2-allyl-3,4,5-tris-benzyloxy-6-benzyloxymethyl-tetrahydro-pyran (1.51b)



(2*S*, 3*R*, 4*S*, 5*R*, 6*R*)-3,4,5-Tris-benzyloxy-6-benzyloxymethyl-tetrahydro-pyran-2-ol (2.78)¹⁴ A stirred solution of methyl- α -D-glucopyranose **2.77** (1 g, 5.15 mmol, 1 equiv) and potassium hydroxide powder (5 g, 89.3 mmol, 17.0 equiv) in dioxane (100 mL) was slowly warmed to boiling point. During this time, freshly distilled benzyl chloride (2.75 g, 21.7 mmol, 4.20 equiv) was added dropwise. Once the solution had reached boiling point further benzyl chloride (2.75 g, 21.7 mmol, 4.2 equiv) was added dropwise, and heating was continued for 2 h. The reaction mixture was cooled and the solvent was removed by rotary evaporation under high vacuum pressure. The residue was dissolved in water (50 mL) and ethyl acetate (50 mL), and the aqueous layer washed twice with ethyl acetate (50 mL). The organic layers were combined, washed twice with water, separated, and then the organic layer was treated with glacial acetic acid (43 mL) and 4 M aqueous sulfuric acid (24 mL) and heated to reflux then stirred overnight. The solution was cooled and a white precipitate formed. Water (11 mL) was added, the solution was cooled on ice, and the solid product filtered and washed successively with 1 M aqueous acetic acid (6 mL), water (6 mL) and aqueous methanol (75% v/v, 6 mL). The crude product was purified by flash chromatography on silica (ethyl acetate/DCM, 1:9) to give **2.78** (795 mg, 29%) as a white solid.

mp = 146-148°C

¹H NMR (CDCl₃, 500 MHz) δ 3.57-3.72 (m, 4H, sugar moiety), 3.96 (t, J = 9.3 Hz, 1H, sugar moiety), 4.03 (m, 1H, sugar moiety), 4.47-4.95 (m, 8H, CH₂Ph), 5.22 (d, J = 3.5 Hz, 1H, H-C_{anomeric}), 7.13-7.35 (m, 20H, ArH).

^{13}C NMR (CDCl_3 , 75 MHz) δ 68.48, 69.93, 72.91, 73.25, 74.85, 75.57, 77.68, 79.79, 81.61, 91.02, 127.46, 127.54, 127.57, 127.73, 127.84, 127.89, 127.96, 128.24, 128.32, 137.62, 137.79, 138.05, 138.56.

(3R, 4S, 5R, 6R)-Acetic acid 3,4,5-tris-benzyloxy-6-benzyloxymethyl-tetrahydro-pyran-2-yl ester (2.79)¹⁵ To a solution of **2.78** (700 mg, 1.29 mmol, 1 equiv) in pyridine (7 mL) was added acetic anhydride (660 mg, 6.47 mmol, 5 equiv). The solution was stirred overnight, poured into chloroform (20 mL), and washed with water (15 mL) and saturated aqueous sodium bicarbonate (20 mL). The resulting organic layer was dried (MgSO_4), and the solvent was removed by evaporation under reduced pressure. The crude product was purified by flash chromatography on silica (ethyl acetate/petroleum ether, 3:7) to give **2.79** (756 mg, 97%) as a colourless, sticky oil and a mixture of isomers (1*:3, 2R*:2S) at the anomeric carbon.

^1H NMR (CDCl_3 , 500 MHz) δ 2.04* (s, 3/4H, COCH_3), 2.12 (s, 9/4H COCH_3), 3.55-3.96* (m, 6H, sugar moiety), 4.46-4.96* (m, 8H, CH_2Ph), 5.60* (d, $J = 8.2$ Hz, 1/4H, $\text{H-C}_{\text{anomeric}}$), 6.35 (d, $J = 3.5$ Hz, 3/4H, $\text{H-C}_{\text{anomeric}}$), 7.12-7.33* (m, 20H, ArH). (*Denotes minor 2R isomer).

^{13}C NMR (CDCl_3 , 75 MHz) δ (mixture of 2R and 2S isomers, peaks not assigned) 20.84, 20.89, 67.89, 72.63, 72.97, 73.27, 73.31, 74.77, 74.80, 75.06, 75.24, 75.46, 76.74, 78.66, 80.83, 81.45, 84.56, 89.78, 93.83, 127.46, 127.50, 127.56, 127.64, 127.76, 127.93, 128.07, 128.21, 128.28, 137.40, 137.59, 137.66, 137.82, 137.91, 138.17, 138.43, 169.09, 169.22.

(2R, 3R, 4S, 5R, 6R)-2-Allyl-3,4,5-tris-benzyloxy-6-benzyloxymethyl-tetrahydro-pyran (1.51b)¹⁶ To a stirred solution of acetyl derivative **2.79** (480 mg, 0.80 mmol, 1 equiv) in freshly distilled acetonitrile (6 mL) under an inert atmosphere was added allyltrimethyl silane (182 mg, 1.60 mmol, 2 equiv) and boron trifluoride diethyl etherate (454 mg, 3.2 mmol, 4 equiv) at 4°C. The mixture was stirred under an inert atmosphere at 4°C for 24 h, then poured into a saturated aqueous sodium bicarbonate solution and extracted twice with DCM. The resulting organic layer was dried (MgSO_4), and the solvent was removed by evaporation under reduced pressure. The crude product was purified by flash chromatography on silica (ethyl acetate/petroleum ether, 1:4) to give **1.51b** (228 mg, 50%) as a white solid and a single anomer.

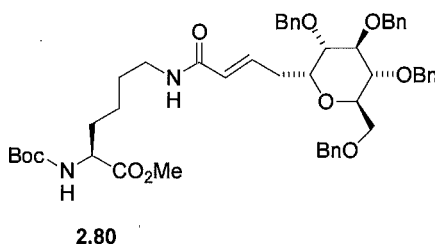
mp = 63-64°C

^1H NMR (CDCl_3 , 500 MHz) δ 2.52 (m, 2H, $\text{CH}_2\text{CH}=\text{CH}_2$), 3.62-3.85 (m, 6H, sugar moiety), 4.17 (m, 1H, $\text{H-C}_{\text{anomeric}}$), 4.49-4.98 (m, 8H, CH_2Ph), 5.10 (dd, $J = 2.0, 10.3$ Hz, 1H, $\text{CH}=\text{CHH}$), 5.14 (dd, $J = 2.0, 17.1$ Hz, 1H, $\text{CH}=\text{CHH}$), 5.85 (m, 1H, $\text{CH}=\text{CH}_2$), 7.15-7.38 (m, 20H, ArH).

^{13}C NMR (CDCl_3 , 75 MHz) δ 29.58, 68.63, 70.87, 72.78, 73.18, 73.42, 74.80, 75.17, 77.84, 79.77, 82.14, 116.68, 127.33, 127.37, 127.46, 127.53, 127.56, 127.60, 127.70, 128.07, 128.13, 128.17, 134.50, 137.81, 137.97, 138.51.

Micro. Calcd for $\text{C}_{37}\text{H}_{40}\text{O}_5$: C, 78.72; H, 7.09. Found: C, 78.53; H, 7.00.

(2S)-(2R, 3R, 4S, 5R, 6R)-2-tert-Butoxycarbonylamino-6-[4-(3,4,5-tris-benzyloxy-6-benzyloxymethyl-tetrahydro-pyran-2-yl)-but-2-enoylamino]-hexanoic acid methyl ester (2.80)



N_ϵ -Acryloyl lysine **2.37** (10 mg, 0.03 mmol, 1 equiv) was reacted with **1.51b** (54 mg, 0.10 mmol, 3 equiv) and Grubbs second generation catalyst **1.17** (5 mg, 0.01 mmol, 0.2 equiv) according to General Procedure X. The crude product was purified by chromatography on silica (petroleum ether/ethyl acetate, 1:4) to give **2.80** (15 mg, 55%) as a pinky, white solid.

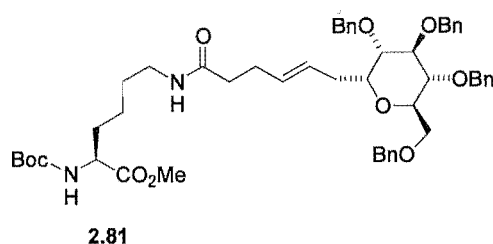
mp = 125-128°C

^1H NMR (CDCl_3 , 500 MHz) δ 1.34 (m, 2H, CHCH_2CH_2), 1.43 (s, 9H, $\text{C}(\text{CH}_3)_3$), 1.48 (m, 2H, $\text{CH}_2\text{CH}_2\text{NH}$), 1.63 (m, 1H, NHCHCHH), 1.78 (m, 1H, NHCHCHH), 2.58 (m, 2H, $=\text{CHCH}_2$), 3.22 (m, 2H, CH_2NH), 3.59-3.76 (m br, 6H, sugar moiety), 3.72 (s, 3H, OCH_3), 4.16 (m, 1H, $\text{H-C}_{\text{anomeric}}$), 4.27 (m br, 1H, NHCH), 4.44-4.92 (m, 8H, CH_2Ph), 5.09 (d br, $J = 7.3$ Hz, 1H, CHNH), 5.46 (s br, 1H, CH_2NH), 5.85 (d, $J = 15.1$ Hz, 1H, $\text{COCH}=\text{}$), 6.78 (dt, $J = 6.8, 14.9$ Hz, 1H, $=\text{CHCH}_2$), 7.12-7.34 (m, 20H, ArH).

^{13}C NMR (CDCl_3 , 75 MHz) δ 22.53, 27.70, 28.26, 28.93, 32.32, 38.99, 52.24, 53.08, 68.93, 71.35, 73.09, 73.18, 73.44, 75.01, 75.38, 77.85, 79.65, 79.84, 82.14, 125.80, 127.59, 127.61, 127.72, 127.78, 127.81, 127.84, 127.89, 128.31, 128.35, 128.42, 137.93, 137.98, 138.52, 140.04, 155.45, 165.68, 173.19.

HRMS ($\text{M}+\text{Na}$) Found 873.4378 (Calcd for $\text{C}_{50}\text{H}_{62}\text{N}_2\text{O}_{10}\text{Na}$ 873.4380).

(2*S*)-(2*R*, 3*R*, 4*S*, 5*R*, 6*R*)-2-*tert*-Butoxycarbonylamino-6-[6-(3,4,5-tris-benzyloxy-6-benzyloxymethyl-tetrahydro-pyran-2-yl)-hex-4-enoylamino]-hexanoic acid methyl ester (2.81)



*N*_ε-Pentenoyl lysine **2.35** (50 mg, 0.15 mmol, 1 equiv) was reacted with **1.51b** (247 mg, 0.44 mmol, 3 equiv) and Grubbs second generation catalyst **1.17** (25 mg, 0.03 mmol, 0.2 equiv) according to General Procedure X. The crude product was purified by chromatography on silica (petroleum ether/ethyl acetate, 3:7) to give a mixture (55 mg, 43%) of **2.81** and the double bond migrated product **2.81a** as a brown oil (6:1 by ^1H NMR).

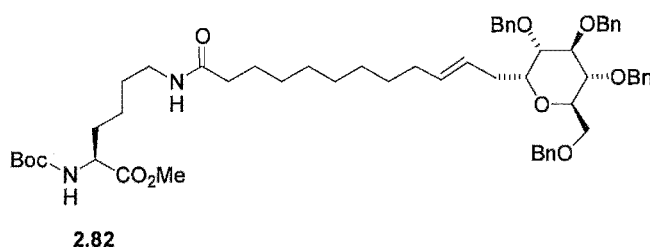
^1H NMR (CDCl_3 , 500 MHz) from mixture **2.81** δ 1.25-1.48 (m, 4H, CH_2), 1.43 (s, 9H, $(\text{CH}_3)_3$), 1.64 (m, 1H, NHCHCHH), 1.77 (m, 1H, NHCHCHH), 2.07 (m, 2H, COCH_2), 2.27 (m, 2H, COCH_2CH_2), 2.40 (m, 2H, $=\text{CHCH}_2\text{CH}$), 3.15 (br m, 2H, CH_2NH), 3.48-3.80 (m, 6H, sugar moiety), 3.72 (s, 3H, OCH_3), 4.01 (m, 1H, $\text{H-C}_{\text{anomeric}}$), 4.26 (br s, 1H, NHCH), 4.44-4.94 (m, 8H, CH_2Ph), 5.07 (d br, $J = 7.8$ Hz, 1H, NHCH), 5.45 (m br, 2H, HC=CH), 5.65 (s br, 1H, CH_2NH), 7.11-7.32 (m, 20H, ArH).

Selected data ^1H NMR (CDCl_3 , 500 MHz) from mixture **2.81a** δ 2.89 (d, $J = 4.5$ Hz, $\text{COCH}_2\text{CH=}$), 5.58 (m, CH=CH), 5.77 (m, CH_2NH).

^{13}C NMR (CDCl_3 , 75 MHz) from mixture **2.81** δ 22.34, 28.09, 28.15, 28.53, 28.79, 31.86, 36.13, 38.59, 51.98, 53.013, 69.27, 70.82, 72.74, 72.88, 73.15, 73.20, 73.46, 74.81, 75.16, 78.08, 79.53, 79.62, 79.79, 82.06, 126.90, 127.35, 127.52, 127.59, 127.68, 127.73, 128.13, 130.99, 137.60, 137.81, 137.94, 138.44, 155.31, 172.41, 173.02.

HRMS ($\text{M}+\text{H}$) Found 879.4798 (Calcd for $\text{C}_{52}\text{H}_{67}\text{N}_2\text{O}_{10}$ 879.4796).

(2S)-(2R, 3R, 4S, 5R, 6R)-2-tert-Butoxycarbonylamino-6-[12-(3,4,5-tris-benzyloxy-6-benzyloxymethyl-tetrahydro-pyran-2-yl)-dodec-10-enoylamino]-hexanoic acid methyl ester (2.82)



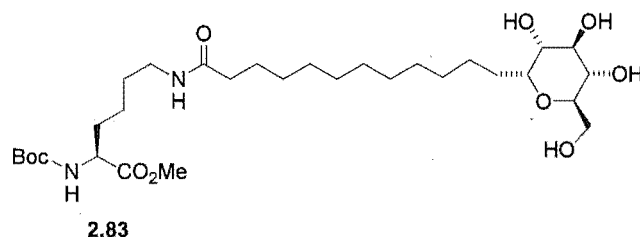
*N*_ε-Decenoyl lysine **2.34** (15 mg, 0.04 mmol, 1 equiv) was reacted with **1.51b** (60 mg, 0.12 mmol, 3 equiv) and Grubbs second generation catalyst **1.17** (6 mg, 0.01 mmol, 0.2 equiv) according to General Procedure X. The crude product was purified by chromatography on silica (petroleum ether/ethyl acetate, 1:1) to give **2.82** (22 mg, 65%) as a brown oil.

^1H NMR (CDCl_3 , 500 MHz) δ 1.30-1.84 (m, 18H, CH_2), 1.49 (s, 9H, $\text{C}(\text{CH}_3)_3$), 2.09 (m, 2H, $=\text{CHCH}_2\text{CH}_2$), 2.17 (m, 2H, COCH_2), 2.47 (m, 2H, $=\text{CHCH}_2\text{CH}$), 3.27 (m, 2H, CH_2NH), 3.65-3.85 (m, 6H, sugar moiety), 3.78 (s, 3H, OCH_3), 4.12 (m, 1H, $\text{H}-\text{C}_{\text{anomeric}}$), 4.32 (s br, 1H, NHCH), 4.50-5.00 (m, 8H, CH_2Ph), 5.13 (d br, $J = 7.4$ Hz, 1H, NHCH), 5.43 (dt, $J = 6.3, 15.7$ Hz, 1H, $\text{CH}=\text{CH}$), 5.52 (dt, $J = 6.4, 15.6$ Hz, 1H, $\text{CH}=\text{CH}$), 5.59 (br s, 1H, CH_2NH), 7.16-7.37 (m, 20H, ArH).

^{13}C NMR (CDCl_3 , 75 MHz) δ 22.52, 25.73, 28.28, 28.51, 29.00, 29.09, 29.30, 29.42, 32.41, 32.62, 36.80, 38.96, 52.27, 53.08, 68.89, 70.93, 72.92, 73.38, 73.97, 75.04, 75.38, 78.10, 79.79, 80.14, 82.38, 125.63, 125.85, 127.53, 127.57, 127.69, 127.75, 127.83, 127.86, 127.94, 128.27, 128.36, 129.71, 132.96, 138.05, 138.15, 138.23, 138.73, 155.47, 173.14, 173.20.

HRMS ($\text{M}+\text{H}$) Found 963.5748 (Calcd for $\text{C}_{58}\text{H}_{79}\text{N}_2\text{O}_{10}$ 963.5735).

(2*S*)-(2*R*, 3*R*, 4*S*, 5*R*, 6*R*)-2-*tert*-Butoxycarbonylamino-6-[12-(3,4,5-trihydroxy-6-hydroxymethyl-tetrahydro-pyran-2-yl)-dodecanoylamino]-hexanoic acid methyl ester (2.83)



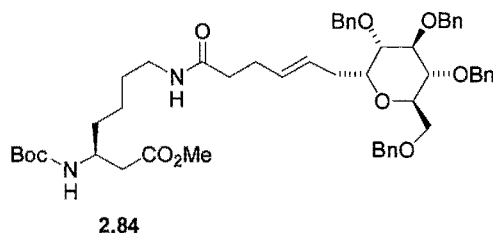
Compound **2.82** (12 mg, 0.012 mmol) was dissolved in ethanol (20 mL) and 10 % palladium/carbon (5 mg) was added. The mixture was placed in a Parr hydrogenator and stirred under an atmosphere of H₂ at 74 PSI for 20 h. The solution was filtered through celite to remove the catalyst, and the resulting organic solvent was removed by evaporation under reduced pressure to give **2.83** (8 mg, quantitative) as a sticky, yellow oil.

¹H NMR (CD₃OD, 500 MHz) δ 1.34-1.80 (m, 22H, CH₂), 1.47 (s, 9H, C(CH₃)₃), 2.20 (t, *J* = 7.3 Hz, 2H, COCH₂), 3.19 (t, *J* = 6.8 Hz, 2H, CH₂NH), 3.27-3.81 (m, 6H, sugar moiety), 3.74 (s, 3H, OCH₃), 3.90 (4.12 m, 1H, H-C_{anomeric}), 4.11 (m, 1H, NHCH).

¹³C NMR (CD₃OD, 75 MHz) δ 24.54, 25.69, 26.92, 27.38, 28.25, 29.01, 30.22, 30.59, 30.71, 30.90, 31.00, 32.53, 37.45, 40.24, 52.88, 55.28, 63.40, 63.66, 72.71, 73.42, 74.61, 75.54, 77.53, 80.84, 158.45, 175.27, 176.58.

HRMS (M+H) Found 605.4012 (Calcd for C₃₀H₅₇N₂O₁₀ 605.4013).

3-*tert*-Butoxycarbonylamino-7-[6-(3,4,5-tris-benzyloxy-6-benzyloxymethyl)-tetrahydro-pyran-2-yl]-hex-4-enoylamino]-heptanoic acid methyl ester (2.84)



*N*_ε-pentenoyl-β-Lysine **2.43** (54 mg, 0.15 mmol, 1 equiv) was reacted with **1.51b** (170 mg, 0.5 mmol, 2 equiv) and Grubbs second generation catalyst **1.17** (26 mg, 0.02 mmol, 0.2

equiv) according to General Procedure X. The crude product was purified by chromatography on silica (petroleum ether/ethyl acetate, 1:4) to give a mixture (46 mg, 34%) of **2.84** and double bond migrated product **2.84a** as a brown oil (3:1 by ^1H NMR).

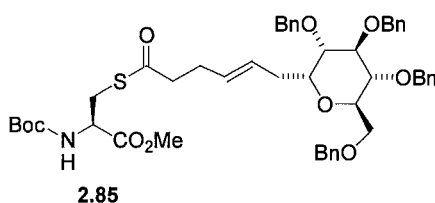
^1H NMR (CDCl_3 , 500 MHz) from mixture **2.84** δ 1.29-1.50 (m, 6H, CH_2), 1.42 (s, 9H, $\text{C}(\text{CH}_3)_3$), 1.65 (m, 2H, CH_2), 2.08 (m, 2H, COCH_2CH_2), 2.28 (m, 2H, COCH_2CH_2), 2.39 (m, 2H, CHCH_2CO), 2.49 (m, 2H, $=\text{CHCH}_2\text{CH}$), 3.15 (m, 2H, CH_2N), 3.49-3.79 (m, 6H, sugar moiety), 3.66 (s, 3H, OCH_3), 3.87 (m, 1H, NHCH), 4.02 (m, 1H, $\text{H-C}_{\text{anomeric}}$), 4.44-4.93 (m, 9H, CH_2Ph , NHCH), 5.45 (m, 2H, $\text{HC}=\text{CH}$), 5.66 (br s, 1H, CH_2NH), 7.11-7.31 (m, 20H, ArH).

Selected data ^1H NMR (CDCl_3 , 500 MHz) from mixture **2.84a** δ 2.90 (d, $J = 4.4$ Hz, $\text{COCH}_2\text{CH}=\text{CH}$), 5.58 (m, $\text{CH}=\text{CH}$), 5.77 (m, CH_2NH).

^{13}C NMR (CDCl_3 , 75 MHz) from mixture **2.84** δ 23.05, 28.23, 28.62, 28.98, 33.89, 36.25, 38.78, 39.04, 40.30, 47.17, 51.47, 69.31, 70.90, 72.86, 73.29, 73.57, 74.90, 75.25, 77.87, 78.15, 79.89, 82.16, 126.95, 127.41, 127.56, 138.52, 127.59, 127.67, 127.76, 127.80, 128.20, 131.05, 137.70, 137.90, 138.03, 155.34, 171.85, 172.42.

HRMS ($\text{M}+\text{H}$) Found 893.4959 (Calcd for $\text{C}_{53}\text{H}_{69}\text{N}_2\text{O}_{10}$ 893.4952).

(2R)-(2R, 3R, 4S, 5R, 6R)-2-tert-Butoxycarbonylamino-3-[6-(3,4,5-tris-benzyloxy-6-benzyloxymethyl-tetrahydro-pyran-2-yl)-hex-4-enoylsulfanyl]-propionic acid methyl ester (2.85)



S-Pentenoyl cysteine **2.49a** (20 mg, 0.06 mmol, 1 equiv) was reacted with **1.51b** (107 mg, 0.19 mmol, 3 equiv) and Grubbs second generation catalyst **1.17** (11 mg, 0.01 mmol, 0.2 equiv) according to General Procedure X. The crude product was purified by chromatography on silica (ethyl acetate/petroleum ether, 1:4) to give a mixture (23 mg, 43%) of **2.85** and a double bond migrated product **2.85a** as a brown oil (4:1 by ^1H NMR).

^1H NMR (CDCl_3 , 500 MHz) from mixture **2.85** δ 1.49 (s, 9H, CH_3), 2.42 (m, 4H, $\text{CH}_2\text{CH}=\text{CHCH}_2$), 2.63 (t, $J = 7.6$ Hz, 2H, COCH_2), 3.39 (m br, 2H, CH_2S), 3.64-3.83 (m, 6H, sugar moiety), 3.78 (s, 3H, OCH_3), 4.11 (m, 1H, $\text{H-C}_{\text{anomeric}}$), 4.50-4.98 (m, 9H, CH_2Ph , NHCH), 5.31 (d br, $J = 7.5$ Hz, 1H, NH), 5.51 (dq, $J = 5.4, 15.6$ Hz, 2H, $\text{CH}=\text{CH}$), 7.16-7.37 (m, 20H, ArH).

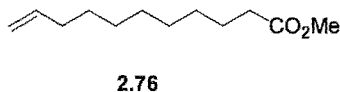
Selected data ^1H NMR (CDCl_3 , 500 MHz) from mixture **2.85a** δ 2.90 (d, $J = 4.3$ Hz, $\text{COCH}_2\text{CH}=\text{CH}$), 5.58 (m, $\text{CH}=\text{CH}$), 5.77 (m, NH).

^{13}C NMR (CDCl_3 , 75 MHz) from mixture **2.85** δ 28.21, 28.39, 30.92, 43.62, 52.57, 52.98, 68.84, 70.98, 72.96, 73.36, 73.77, 74.99, 75.34, 78.03, 79.97, 80.12, 82.27, 127.51, 127.58, 127.65, 127.71, 127.78, 127.84, 127.89, 128.26, 128.31, 128.34, 129.72, 137.93, 138.06, 138.13, 138.64, 154.99, 170.88, 197.63.

HRMS ($\text{M}+\text{H}$) Found 854.3926 (Calcd for $\text{C}_{49}\text{H}_{60}\text{NO}_{10}\text{S}$ 854.3938).

5.2.4 Synthesis of Fatty Acid CM compounds

Undec-10-enoic acid methyl ester (**2.76**)

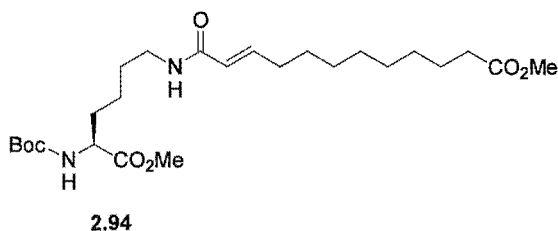


To a solution of 10-decenoic acid **2.75** (1 g, 5.43 mmol) in methanol (25 mL) was added Amberlyst 15 IER resin (870 mg). The solution was stirred overnight, filtered to remove the resin, and the solvent was removed by evaporation under reduced pressure. The crude product was purified by flash chromatography on silica (ethyl acetate/petroleum ether, 1:19) to give **2.76** (803 mg, 75%) as a colourless liquid.

^1H NMR (CDCl_3 , 500 MHz) δ 1.27 (m, 8H, CH_2), 1.35 (m, 2H, $\text{CH}=\text{CH}_2\text{CH}_2$), 1.60 (m, 2H, $\text{CH}_2\text{CH}_2\text{CO}$), 2.02 (q, $J = 7.3$ Hz, 2H, $=\text{CHCH}_2\text{CH}_2$), 2.29 (t, $J = 7.6$ Hz, 2H, COCH_2), 3.65 (s, 3H, OCH_3), 4.91 (dd, $J = 1.5, 10.3$ Hz, 1H, $\text{CH}=\text{CHH}$), 4.97 (ddd, $J = 1.5, 3.4, 17.1$ Hz, 1H, $\text{CH}=\text{CHH}$), 5.79 (m, 1H, $\text{CH}=\text{CH}_2$).

^{13}C NMR (CDCl_3 , 75 MHz) δ 24.75, 28.71, 28.87, 28.95, 29.03, 29.11, 33.60, 33.84, 51.12, 113.94, 138.82, 173.91.

(5*S*)-11-(5-*tert*-Butoxycarbonylamino-5-methoxycarbonyl-pentylcarbamoyl)-undec-10-enoic acid methyl ester (**2.94**)



N_ϵ -Acryloyl lysine **2.37** (20 mg, 0.06 mmol, 1 equiv) was reacted with **2.76** (38 mg, 0.20 mmol, 3 equiv) and Grubbs second generation catalyst **1.17** (11 mg, 0.01 mmol, 0.2 equiv) according to General Procedure X. The crude product was purified by chromatography on silica (petroleum ether/ethyl acetate, 3:7) to give a mixture (21 mg, 68%) of **2.94** and double bond migrated product **2.94a** as a brown oil (12:1 by ^1H NMR).

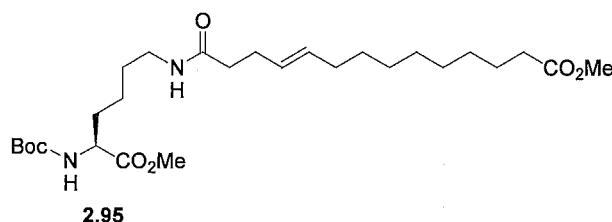
^1H NMR (CDCl_3 , 500 MHz) from mixture **2.94** δ 1.25-1.41 (m, 12H, CH_2), 1.41 (s, 9H, $(\text{CH}_3)_3$), 1.48-1.65 (m, 5H, $\text{CH}_2\text{CH}_2\text{NH}$, $\text{CH}_2\text{CH}_2\text{CO}$, NHCHCHH), 1.77 (m, 1H, NHCHCHH), 2.12 (m, 2H, $=\text{CHCH}_2$), 2.26 (t, $J = 7.6$ Hz, 2H, CH_2CO), 3.27 (q, $J = 6.3$ Hz, 2H, CH_2NH), 3.63 (s, 3H, OCH_3), 3.70 (s, 3H, OCH_3), 4.24 (m br, 1H, NHCH), 5.13 (d br, $J = 7.8$ Hz, 1H, NHCH), 5.73 (m br, 2H, CH_2NH , COCH=), 6.78 (dt, $J = 6.8, 15.6$ Hz, 1H, $=\text{CHCH}_2$).

Selected data ^1H NMR (CDCl_3 , 500 MHz) from mixture **2.94a** δ 2.89 (d, $J = 7.0$ Hz, $\text{COCH}_2\text{CH=}$), 3.19 (m, CH_2NH), 5.51 (m, CH=CH).

^{13}C NMR (CDCl_3 , 75 MHz) from mixture **2.94** δ 22.48, 24.75, 28.08, 28.19, 28.77, 28.86, 28.92, 28.97, 29.02, 31.83, 32.25, 33.93, 38.87, 51.33, 52.15, 53.03, 79.72, 123.47, 144.45, 155.45, 166.11, 173.12, 174.20.

HRMS ($\text{M}+\text{H}$) Found 485.3224 (Calcd for $\text{C}_{25}\text{H}_{45}\text{N}_2\text{O}_7$ 485.3227).

(5S)-13-(5-*tert*-Butoxycarbonylamino-5-methoxycarbonyl-pentylcarbamoyl)-tridec-10-enoic acid methyl ester (2.95)



N_ϵ -Pentenoyl lysine **2.35** (50 mg, 0.15 mmol, 1 equiv) was reacted with **2.76** (87 mg, 0.44 mmol, 3 equiv) and Grubbs second generation catalyst **1.17** (25 mg, 0.03 mmol, 0.2 equiv) according to General Procedure X. The crude product was purified by chromatography on silica (petroleum ether/ethyl acetate, 3:7) to give a mixture (36 mg, 48%) of **2.95** and double bond migrated isomers **2.95a** and **b** as a brown oil (34:4:1 by ^1H NMR).

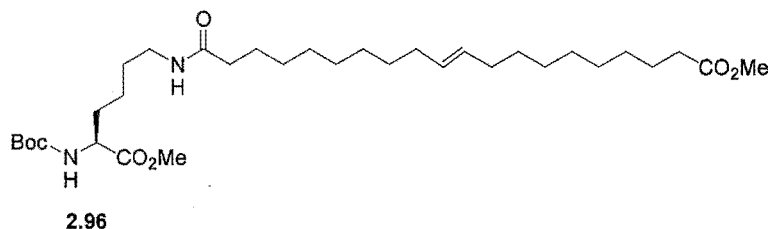
^1H NMR (CDCl_3 , 500 MHz) from mixture **2.95** δ 1.22-1.38 (m, 12H, CH_2), 1.39 (s, 9H, $(\text{CH}_3)_3$), 1.47 (m, 1H, $\text{CH}_2\text{CH}_2\text{NH}$), 1.58 (m br, 5H, $\text{CH}_2\text{CH}_2\text{CO}$, NHCHCHH), 1.74 (m, 1H, NHCHCHH), 1.91 (m, 2H, $=\text{CHCH}_2(\text{CH}_2)_7$), 2.17 (m, 2H, $\text{COCH}_2\text{CH}_2=\text{CH}$), 2.26 (m, 4H, COCH_2), 3.18 (m, 2H, CH_2NH), 3.62 (s, 3H, OCH_3), 3.69 (s, 3H, OCH_3), 4.22 (m br, 1H, NHCH), 5.12 (d br, $J = 7.9$ Hz, 1H, NHCH), 5.37 (dt, $J = 6.3, 14.1$ Hz, 1H, CH=CH), 5.45 (dt, $J = 6.5, 14.1$ Hz, 1H, CH=CH), 5.77 (s br, 1H, CH_2NH).

Selected data ^1H NMR (CDCl_3 , 500 MHz) from mixture **2.95a** and **b** δ 2.91 (d, $J = 6.9$ Hz, $\text{COCH}_2\text{CH=}$), 2.98 (d, $J = 7.6$ Hz, $\text{COCH}_2\text{CH=}$), 3.30 (m, CH_2NH), 5.51 (m, CH=CH), 5.65 (m, CH_2NH).

^{13}C NMR (CDCl_3 , 75 MHz) from mixture **2.95** δ 22.35, 24.67, 28.07, 28.45, 28.77, 28.83, 28.93, 28.99, 29.05, 29.16, 31.95, 32.23, 32.82, 36.36, 38.65, 51.20, 52.00, 53.00, 79.54, 128.16, 131.44, 155.36, 172.55, 173.03, 174.11.

HRMS ($\text{M}+\text{H}$) Found 513.3535 (Calcd for $\text{C}_{27}\text{H}_{49}\text{N}_2\text{O}_7$ 513.3540).

(5S)-19-(5-*tert*-Butoxycarbonylamino-5-methoxycarbonyl-pentylcarbamoyl)-nonadec-10-enoic acid methyl ester (2.96**)**



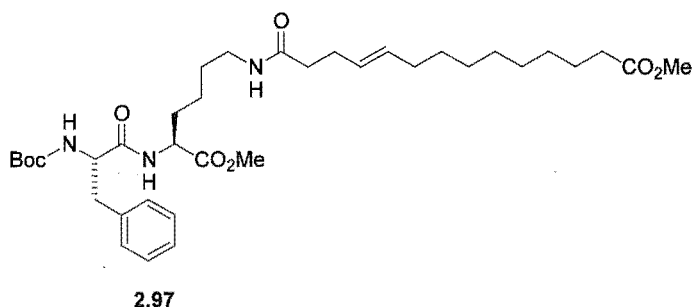
N_ϵ -Decenoyl lysine **2.34** (20 mg, 0.05 mmol, 1 equiv) was reacted with **2.76** (29 mg, 0.14 mmol, 3 equiv) and Grubbs second generation catalyst **1.17** (8 mg, 0.01 mmol, 0.2 equiv) according to General Procedure X. The crude product was purified by chromatography on silica (petroleum ether/ethyl acetate, 1:4) to give **2.96** (18 mg, 64%) as a brown oil.

^1H NMR (CDCl_3 , 500 MHz) δ 1.25-1.38 (m br, 24H, CH_2), 1.41 (s, 9H, $\text{C}(\text{CH}_3)_3$), 1.48 (m, 2H, $\text{CH}_2\text{CH}_2\text{NH}$), 1.57-1.64 (m br, 5H, COCH_2CH_2 , NHCHCHH), 1.77 (m, 1H, NHCHCHH), 1.95 (m, 4H, $\text{CH}_2\text{CH=CH}_2$), 2.12 (t, $J = 7.5$ Hz, 2H, COCH_2), 2.27 (t, $J = 7.3$ Hz, 2H, COCH_2), 3.20 (m, CH_2NH), 3.63 (s, 3H, OCH_3), 3.71 (s, 3H, OCH_3), 4.24 (m br, 1H, NHCH), 5.12 (d br, $J = 7.7$ Hz, 1H, CHNH), 5.34 (m, 2H, CH=CH), 5.72 (s br, 1H, CH_2NH).

^{13}C NMR (CDCl_3 , 75 MHz) δ 22.49, 24.85, 25.72, 27.09, 28.23, 28.97, 29.03, 29.12, 29.19, 29.26, 28.51, 29.49, 29.62, 32.31, 32.48, 34.01, 36.73, 38.89, 51.36, 52.20, 53.06, 79.79, 129.76, 130.22, 155.45, 173.15, 173.21, 174.27.

HRMS ($\text{M}+\text{H}$) Found 597.4472 (Calcd for $\text{C}_{33}\text{H}_{61}\text{N}_2\text{O}_7$ 597.4479).

(2*S*,5*S*)-13-[5-(2-*tert*-Butoxycarbonylamino-3-phenyl-propionylamino)-5-methoxy-carbonyl-pentylcarbamoyl]-tridec-10-enoic acid methyl ester (2.97**)**



*N*_e-Pentenoyl dipeptide **2.54** (20 mg, 0.04 mmol, 1 equiv) was reacted with **2.76** (24 mg, 0.12 mmol, 3 equiv) and Grubbs second generation catalyst **1.17** (7 mg, 0.01 mmol, 0.2 equiv) according to General Procedure X. The crude product was purified by chromatography on silica (petroleum ether/ethyl acetate, 1:4) to give a mixture (11 mg, 41%) of **2.97** and double bond migrated product **2.97a** as a brown oil (4:1 by ¹H NMR).

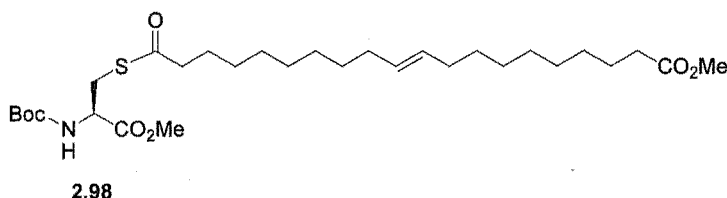
¹H NMR (CDCl₃, 500 MHz) from mixture **2.97** δ 1.31-1.51 (m, 14H, CH₂), 1.43 (s, 9H, (CH₃)₃), 1.67 (m, 3H, CH₂CH₂NH, NHCHCHH), 1.84 (m, 1H, NHCHCHH), 2.03 (m, 2H, =CHCH₂(CH₂)₇), 2.26 (m, 2H, CH₂CH=CH₂), 2.33 (m, 4H, COCH₂), 3.10 (m br, 2H, CH₂Ph), 3.24 (m, 2H, CH₂NH), 3.70 (s, 3H, OCH₃), 3.73 (s, 3H, OCH₃), 4.41 (m br, 1H, NHCH_{Ph}), 4.55 (m br, 1H, NHCH_{Lys}), 5.19 (s br, 1H, NHCH_{Ph}), 5.41 (dt, *J* = 6.0, 15.4 Hz, 1H, CH=CH), 5.49 (dt, *J* = 6.3, 15.3 Hz, 1H, CH=CH), 5.87 (s br, 1H, CH₂NH), 6.70 (d br, 1H, *J* = 7.5 Hz, NHCH_{Lys}), 7.23-7.33 (m, 5H, ArH).

Selected data ¹H NMR (CDCl₃, 500 MHz) from mixture **2.97a** δ 2.96 (d, *J* = 6.9 Hz, COCH₂CH=), 5.59 (m, CH=CH).

¹³C NMR (CDCl₃, 75 MHz) from mixture **2.97** δ 22.11, 24.87, 28.19, 28.66, 28.91, 28.99, 29.05, 29.14, 29.20, 29.36, 31.68, 32.44, 34.05, 36.61, 38.62, 38.74, 51.42, 51.92, 52.32, 55.69, 80.11, 126.84, 128.27, 128.53, 129.29, 131.76, 136.55, 155.43, 171.28, 172.17, 172.84, 174.32.

HRMS (M+H) Found 660.4208 (Calcd for C₃₆H₅₈N₃O₈ 660.4224).

(2R)-19-(2-*tert*-Butoxycarbonylamino-2-methoxycarbonyl-ethylsulfanylcarbonyl)-nonadec-10-enoic acid methyl ester (2.98)



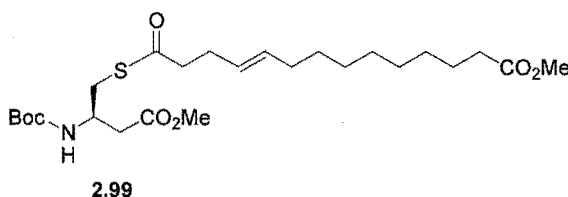
*N*_ε-Decenoyl cysteine **2.48** (30 mg, 0.07 mmol, 1 equiv) was reacted with **2.76** (45 mg, 0.23 mmol, 3 equiv) and Grubbs second generation catalyst **1.17** (13 mg, 0.01 mmol, 0.2 equiv) according to General Procedure X. The crude product was purified by chromatography on silica (ethyl acetate/petroleum ether, 1:4) to give **2.98** (26 mg, 61%) as a brown oil.

¹H NMR (CDCl₃, 500 MHz) δ 1.27 (m, 20H, CH₂), 1.41 (s, 9H, (CH₃)₃), 1.60 (m, 4H, COCH₂CH₂), 1.94 (m, 4H, CH₂CH=CH₂), 2.27 (t, *J* = 7.5 Hz, 2H, OCOCH₂), 2.52 (t, *J* = 7.5 Hz, 2H, SCHOCH₂), 3.31 (m, 2H, SCH₂), 3.64 (s, 3H, OCH₃), 3.72 (s, 3H, OCH₃), 4.49 (m br, 1H, NHCH), 5.22 (d br, *J* = 7.5 Hz, 1H, NH), 5.33 (m, 2H, CH=CH).

¹³C NMR (CDCl₃, 75 MHz) δ 24.83, 25.49, 27.07, 28.17, 28.75, 28.96, 29.00, 29.03, 29.08, 29.11, 29.14, 29.19, 29.43, 29.47, 30.83, 32.46, 33.98, 43.89, 51.32, 52.52, 53.00, 80.00, 130.18, 130.23, 154.97, 170.90, 174.20, 198.39.

HRMS (M+H) Found 572.3625 (Calcd for C₃₀H₅₄NO₇S 572.3621).

(2R)-13-(2-*tert*-Butoxycarbonylamino-3-methoxycarbonyl-propylsulfanylcarbonyl)-tridec-10-enoic acid methyl ester (2.99)



S-Pentenoyl-β-cysteine **2.51** (60 mg, 0.18 mmol, 1 equiv) was reacted with **2.76** (108 mg, 0.54 mmol, 3 equiv) and Grubbs second generation catalyst **1.17** (31 mg, 0.04 mmol, 0.2 equiv) according to General Procedure X. The crude product was purified by

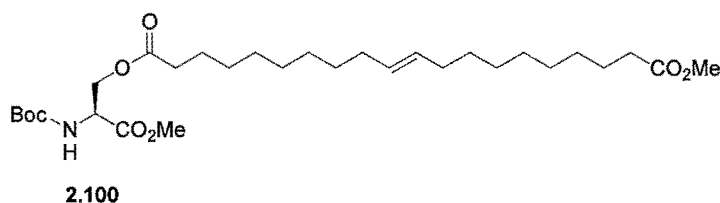
chromatography on silica (ethyl acetate/DCM, 1:19) to give **2.99** (53 mg, 59%) as a brown oil.

^1H NMR (CDCl_3 , 500 MHz) δ 1.25 (m, 12H, CH_2), 1.39 (s, 9H, $\text{C}(\text{CH}_3)_3$), 1.57 (m, 2H, CH_2), 1.92 (m, 2H, $=\text{CHCH}_2$), 2.28 (m, 4H, COCH_2CH_2 , $\text{COCH}_2(\text{CH}_2)_7$), 2.56 (m, 4H, SCOCH_2 , COCH_2CH), 3.10 (m, 2H, SCH_2), 3.63 (s, 3H, OCH_3), 3.66 (s, 3H, OCH_3), 4.04 (m, 1H, NHCH), 5.11 (br d, $J = 7.8$ Hz, 1H, NH), 5.32 (td, $J = 6.5, 14.3$ Hz, 1H, $\text{CH}=\text{CH}$), 5.42 (td, $J = 6.7, 15.2$ Hz, 1H, $\text{CH}=\text{CH}$).

^{13}C NMR (CDCl_3 , 75 MHz) δ 24.77, 28.18, 28.37, 28.88, 28.92, 28.96, 29.04, 29.10, 29.17, 32.28, 33.92, 37.44, 43.82, 47.50, 51.27, 51.64, 79.38, 127.10, 132.11, 154.94, 171.32, 174.12, 198.33.

HRMS ($\text{M}+\text{H}$) Found 502.2836 (Calcd for $\text{C}_{25}\text{H}_{44}\text{NO}_7\text{S}$ 502.2839).

(2*S*)-Icos-10-enedioic acid 2-*tert*-butoxycarbonylamino-2-methoxycarbonyl-ethyl ester methyl ester (2.100**)**



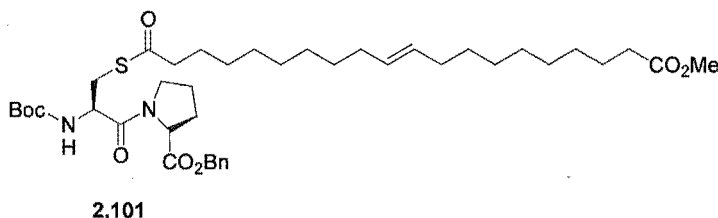
*N*_ε-Decenoyl serine **2.46** (20 mg, 0.05 mmol, 1 equiv) was reacted with **2.76** (31 mg, 0.16 mmol, 3 equiv) and Grubbs second generation catalyst **1.17** (9 mg, 0.01 mmol, 0.2 equiv) according to General Procedure X. The crude product was purified by chromatography on silica (ethyl acetate/petroleum ether, 1:4) to give **2.100** (21 mg, 73%) as a brown oil.

^1H NMR (CDCl_3 , 500 MHz) δ 1.28 (m, 20H, CH_2), 1.44 (s, 9H, $(\text{CH}_3)_3$), 1.59 (m, 4H, COCH_2CH_2), 1.94 (m, 4H, $\text{CH}_2\text{CH}=\text{CH}_2$), 2.28 (m, 4H, COCH_2), 3.65 (s, 3H, OCH_3), 3.75 (s, 3H, OCH_3), 4.30 (dd, $J = 3.6, 11.3$ Hz, 1H, OCHH), 4.44 (dd, $J = 3.6, 11.3$ Hz, 1H, OCHH), 4.55 (m br, 1H, NHCH), 5.27 (d br, $J = 8.2$ Hz, 1H, NH), 5.36 (m, 2H, $\text{CH}=\text{CH}$).

^{13}C NMR (CDCl_3 , 75 MHz) δ 24.73, 24.86, 28.19, 28.64, 28.85, 28.99, 29.02, 29.05, 29.16, 29.21, 29.35, 29.46, 29.51, 32.46, 32.49, 33.89, 34.01, 51.35, 52.61, 52.86, 63.96, 80.20, 130.25, 130.34, 155.06, 170.24, 173.22, 174.23.

HRMS ($\text{M}+\text{H}$) Found 556.3873 (Calcd for $\text{C}_{30}\text{H}_{54}\text{NO}_8$ 556.3849).

1-[2-*tert*-Butoxycarbonylamino-3-(19-methoxycarbonyl-nonadec-10-enoylsulfanyl)-propionyl]-pyrrolidine-2-carboxylic acid benzyl ester (2.101)



*N*_ε-Decenoyl dipeptide **2.57** (50 mg, 0.09 mmol, 1 equiv) was reacted with **2.76** (52 mg, 0.03 mmol, 3 equiv) and Grubbs second generation catalyst **1.17** (15 mg, 0.02 mmol, 0.02 equiv) according to General Procedure X. The crude product was purified by chromatography on silica (ethyl acetate/DCM, 1:9) to give **2.101** (28 mg, 43%) as a brown oil.

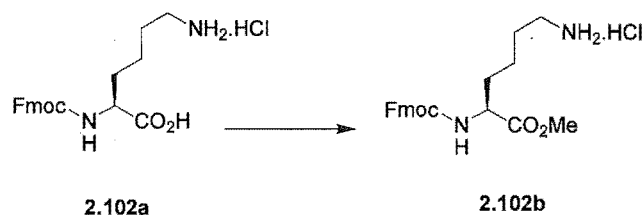
¹H NMR (CDCl₃, 500 MHz) δ 1.26 (m, 18H, CH₂), 1.39 (s, 9H, C(CH₃)₃), 1.61 (m, 4H, CH₂), 1.96 (m, 8H, CH₂), 2.18 (m, 1H, COCHCHH), 2.27 (m, 3H, OCHCH₂, COCHCHH), 2.53 (t, *J* = 7.3 Hz, 2H, SCOCH₂), 2.71 (m, 1H, SCHH), 3.31 (m, 1H, SCHH), 3.63 (s, 3H, OCH₃), 3.72 (m, 1H, NCHH), 3.92 (m, 1H, NCHH), 4.56 (m, 2H, NHCH, NCH), 5.09 (m, 1H, OCHH), 5.17 (m, 1H, OCHH), 5.31 (m, 1H, NH), 5.35 (m, 2H, CH=CH), 7.31 (m, 5H, ArH).

¹³C NMR (CDCl₃, 75 MHz) δ 24.80, 24.85, 25.44, 28.20, 28.83, 28.90, 29.01, 29.04, 29.13, 29.17, 29.20, 29.45, 29.51, 31.74, 32.48, 34.00, 34.42, 43.93, 46.97, 51.34, 58.93, 66.82, 79.67, 125.78, 128.08, 128.21, 128.46, 130.22, 135.45, 155.38, 169.36, 171.33, 174.21, 199.30,

HRMS (M+H) Found 745.4478 (Calcd for C₄₁H₆₅N₂O₈S 745.4462).

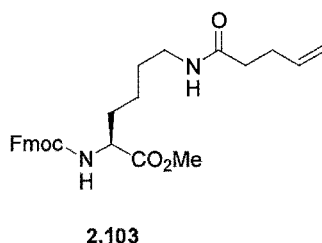
5.2.5 Solid Phase Synthesis

(6S)-6-Amino-2-(9H-fluoren-9-ylmethoxycarbonylamino)-hexanoic acid methyl ester hydrochloride salt (**2.102b**)



Fmoc-*L*-Lys-OH **2.102a** (4 g, 9.9 mmol) was treated with Amberlyst 15 IER (1.6 g) according to General Procedure XV, to give **2.102b** (3.1 g, 75%) as a pale yellow, sticky oil that was used without purification.

(2S)-2-(9H-Fluoren-9-ylmethoxycarbonylamino)-6-(pent-4-enoylamino)-hexanoic acid methyl ester (**2.103**)



Fmoc-*L*-Lys-OMe **2.102b** (503 mg, 1.2 mmol, 1 equiv) and 4-pentenoic acid (113 mg, 1.4 mmol, 1.14 equiv) were treated with EDCI (198 mg, 1.56 mmol, 1.3 equiv) and HOBt (272 mg, 1.8 mmol, 1.5 equiv) according to General Procedure VII. The crude product was purified by flash chromatography on silica (petroleum ether/ethyl acetate, 2:3) to give **2.103** (344 mg, 66%) as a white solid.

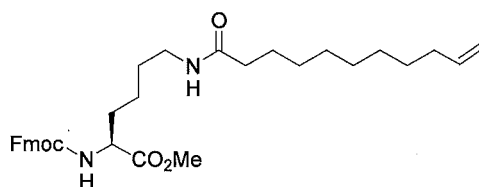
mp = 88-89°C

^1H NMR (CDCl_3 , 500 MHz) δ 1.37 (m, 2H, CH_2), 1.51 (m, 2H, CH_2), 1.70 (m, 1H, NHCHCHH), 1.85 (m, 1H, NHCHCHH), 2.23 (t, $J = 7.4$ Hz, 2H, COCH_2), 2.36 (m, 2H, COCH_2CH_2), 3.23 (m, 2H, NHCH_2), 3.74 (s, 3H, OCH_3), 4.21 (t, $J = 7.0$ Hz, 1H, CH_{Fmoc}), 4.38 (m, 3H, NHCH , $\text{CH}_{2\text{Fmoc}}$), 5.00 (m, 2H, $\text{CH}=\text{CH}_2$), 5.45 (br d, $J = 8.1$ Hz, 1H, NHCH), 5.60 (br s, 1H, NHCH_2), 5.78 (m, 1H, $\text{CH}=\text{CH}_2$).

^{13}C NMR (CDCl_3 , 75 MHz) δ 22.19, 29.34, 31.41, 35.30, 38.49, 46.75, 52.02, 53.43, 66.64, 115.05, 119.64, 124.74, 126.72, 127.38, 136.75, 140.88, 143.36, 143.50, 155.95, 172.40, 172.67.

HRMS ($\text{M}+\text{H}$) Found 465.2389 (Calcd for $\text{C}_{27}\text{H}_{33}\text{N}_2\text{O}_5$ 465.2389).

(2S)-2-(9H-Fluoren-9-ylmethoxycarbonylamino)-6-(undec-10-enoylamino)-hexanoic acid methyl ester (2.104)



2.104

Fmoc-*L*-Lys-OMe **2.102b** (1.5 g, 3.4 mmol, 1 equiv) and 10-decenoic acid (751 mg, 4.1 mmol, 1.14 equiv) were treated with EDCI (844 mg, 4.42 mmol, 1.3 equiv) and HOBt (770 mg, 5.1 mmol, 1.5 equiv) according to General Procedure VII. The crude product was purified by flash chromatography on silica (ethyl acetate/DCM, 2:3) to give **2.104** (1.72 g, 88%) as a white solid.

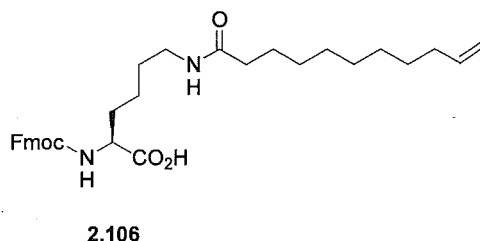
mp = 97-99°C

^1H NMR (CDCl_3 , 500 MHz) δ 1.24 (s, 9H, $\text{C}(\text{CH}_3)_3$), 1.34 (m, 8H, CH_2), 1.52 (m, 4H, CH_2), 1.59 (m, 4H, CH_2), 1.71 (m, 1H, NHCHCHH), 1.85 (m, 1H, NHCHCHH), 2.01 (m, 2H, $\text{CH}_2\text{CH}=\text{CH}_2$), 2.12 (t, $J = 7.6$ Hz, 2H, COCH_2), 3.23 (m, 2H, CH_2NH), 3.74 (s, 3H, OCH_3), 4.22 (t, $J = 7.1$ Hz, 1H, CH_{Fmoc}), 4.38 (m, 3H, NHCH , $\text{CH}_{2\text{Fmoc}}$), 4.91 (m, 1H, $\text{CH}=\text{CHH}$), 4.97 (m, 1H, $\text{CH}=\text{CHH}$), 5.45 (br d, $J = 8.1$ Hz, 1H, NHCH), 5.54 (br s, 1H, CH_2NH), 5.78 (m, 1H, $\text{CH}=\text{CH}_2$), 7.29-7.76 (m, 8H, ArH).

^{13}C NMR (CDCl_3 , 75 MHz) δ 22.22, 25.53, 28.55, 28.68, 28.75, 29.02, 31.49, 33.46, 36.36, 38.48, 46.80, 52.03, 53.44, 66.68, 113.87, 119.66, 124.77, 126.74, 127.40, 138.77, 140.92, 143.38, 143.54, 155.95, 172.67, 173.23.

HRMS (M+H) Found 549.3334 (Calcd for C₃₃H₄₅N₂O₅ 549.3328).

(2S)-2-(9H-Fluoren-9-ylmethoxycarbonylamino)-6-(undec-10-enoylamino)-hexanoic acid (2.106)



*N*_α-Fmoc-*N*_ε-decenoyl-*L*-Lys-OMe **2.104** (508 mg, 0.93 mmol, 1 equiv) was treated with 0.2 M lithium hydroxide (2 equiv) according to General Procedure VIII to give **2.106** (477 mg, 96%) as a yellow solid.

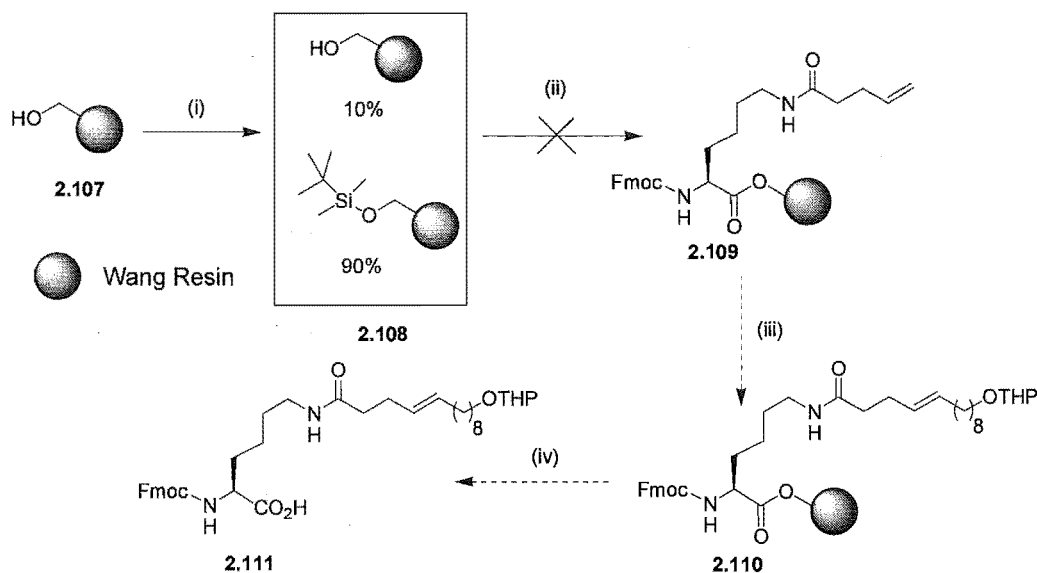
mp = 85-90°C

¹H NMR (CDCl₃, 500 MHz) δ 1.22 (s, 9H, C(CH₃)₃), 1.29-1.57 (m, 16H, CH₂), 1.76 (m, 1H, NHCHCHH), 1.89 (m, 1H, NHCHCHH), 2.00 (m, 2H, CH₂CH=CH₂), 2.14 (t, *J* = 7.5 Hz, 2H, COCH₂), 3.22 (m, 2H, CH₂NH), 4.18 (t, *J* = 7.1 Hz, 1H, CH_{Fmoc}), 4.35 (m, 3H, NHCH, CH_{2Fmoc}), 4.91 (m, 1H, CH=CHH), 4.96 (m, 1H, CH=CHH), 5.78 (m, 1H, CH=CH₂), 5.85 (br d, *J* = 8.0 Hz, 1H, NHCH), 5.99 (br s, 1H, CH₂NH), 7.25-7.73 (m, 8H, ArH).

¹³C NMR (CDCl₃, 75 MHz) δ 22.21, 25.71, 28.74, 28.94, 29.12, 29.14, 29.19, 33.64, 36.41, 39.14, 46.95, 53.45, 66.98, 114.04, 119.83, 124.99, 126.93, 127.59, 128.61, 138.99, 141.09, 143.51, 156.29, 174.60, 174.91.

HRMS (M+H) Found 535.3165 (Calcd for C₃₂H₄₃N₂O₅ 535.3172).

Attempted solid-phase synthesis of 2.111

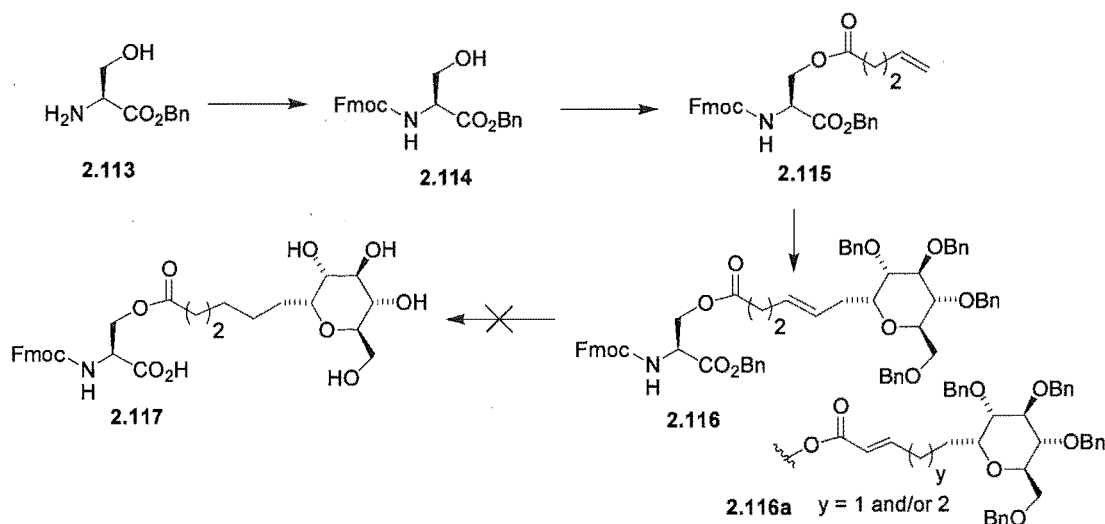


Capping of Wang Resin 2.107. Wang Resin **2.107** (384 mg, ~0.23 mmol) was swelled in DCM (3.5 mL) for 30 min and then treated with *tert*-butyldimethylsilyl chloride (31 mg, 0.21 mmol) and TEA (13 mL). The reaction was shaken for 18 h, filtered, and washed with DCM and ether. The resulting resin (**2.108**) was dried overnight under high vacuum.

Attempted loading of resin 2.108. Capped Wang resin **2.108** (1 eq uncapped binding sites) was swelled in a minimal amount of DCM for 30 min, and then EDCI (8mg, 0.04 mmol, 1.8 eq), DMAP (2mg, 0.02 mmol, 0.7 eq), and **2.106** (18mg, 0.03 mmol, 1.4 eq) in DCM (2 mL) were added and the mixture shaken for 24 hr. The resin was washed with DCM and ether, and dried under high vacuum overnight to give **2.109**.

Attempted cross metathesis coupling. The Wang resin **2.109** was swelled in a minimal amount of DCM for 30 min. A solution of **2.65** (31mg, 0.13 mmol, 4 eq) and catalyst **1.17** (3mg) in DCM was added, and the mixture shaken for 24 hr. The resin was washed with DCM and ether, and dried overnight under high vacuum. The resulting dry resin was swelled in a minimal amount of DCM, and TFA (1.6mL) and DCM (4mL) were added and the mixture shaken for 24 hr. The resin was then washed with DCM and the filtrate collected, and the solvents removed by evaporation under reduced pressure.

Preparation of 6-(3,4,5-tris-benzyloxy-6-benzyloxymethyl-tetrahydro-pyran-2-yl)-hex-4-enoic acid 2-benzyloxycarbonyl-2-(9H-fluoren-9-ylmethoxycarbonylamino)-ethyl ester (2.116)



(2S)-2-(9H-Fluoren-9-ylmethoxycarbonylamino)-3-hydroxy-propionic acid benzyl ester (2.114) L-Ser-OBn.HCl **2.113** (1 g, 4.32 mmol, 1 equiv) was dissolved in DCM (30 mL) and DIPEA (614 mg, 4.75 mmol, 1.1 equiv) was added dropwise at 0°C. After stirring for 10 min a solution of Fmoc-Cl (1.23 g, 4.75 mmol, 1.1 equiv) in DCM (10 mL) was added dropwise and the solution was allowed to warm to rt with stirring for 1 h. The solution was diluted with DCM, and washed once with 1% aqueous sodium bicarbonate, saturated sodium bicarbonate, water, and saturated brine sequentially. The organic layer was dried (MgSO₄) and the solvent removed by evaporation under reduced pressure to give a colourless oil that was recrystallised (petroleum ether/ethyl acetate) to give **2.114** (1.619 g, 90%) as a white solid.

mp = 97-100°C

¹H NMR (CDCl₃, 500 MHz) δ 3.92 (m, 1H, CHHOH), 4.01 (m, 1H, CHHOH), 4.20 (t, *J* = 6.8 Hz, 1H, CH_{Fmoc}), 4.41 (m, 2H, CH_{2Fmoc}), 4.49 (m, 1H, NHCH), 5.21 (s, 2H, OCH₂Ph), 5.78 (br d, *J* = 7.0 Hz, 1H, NH), 7.28-7.76 (m, 13H, ArH).

¹³C NMR (CDCl₃, 75 MHz) δ 46.67, 55.97, 62.53, 66.91, 67.05, 119.65, 124.79, 126.78, 127.40, 127.73, 128.04, 128.23, 134.85, 140.90, 143.33, 156.26, 170.39.

(2S)-Pent-4-enoic acid[2-benzyloxycarbonyl-2-(9H-fluoren-9-ylmethoxycarbonylamino)]-ethyl ester (2.115) Fmoc-L-Ser-OBn **2.114** (1.12 g, 2.7 mmol, 1 equiv) and 4-pentenoic acid (306 mg, 3.1 mmol, 1.14 equiv) were treated with EDCI (562 mg, 2.94 mmol, 1.09 equiv) and DMAP (3 mg, 0.03 mmol, 0.01 equiv) according to the General Procedure XII. The crude product was purified by flash chromatography on silica (ethyl acetate/DCM, 2.5:97.5) to give **2.115** (1.13 g, 94%) as a white solid.

mp = 81-83°C

¹H NMR (CDCl₃, 500 MHz) δ 2.32 (m, 4H, COCH₂CH₂), 4.23 (t, *J* = 7.1 Hz, 1H, CH_{Fmoc}), 4.39 (m, 3H, CH_{2Fmoc}, NHCHCHH), 4.51 (m, 1H, NHCHCHH), 4.67 (m, 1H, NHCH), 5.01 (m, 2H, CH=CH₂), 5.21 (m, 2H, OCH₂Ph), 5.59 (br d, *J* = 8.2 Hz, 1H, NH), 5.77 (m, 1H, CH=CH₂), 7.29-7.77 (m, 13H, ArH). 46.76,

¹³C NMR (CDCl₃, 75 MHz) δ 28.27, 32.69, 53.21, 63.61, 66.91, 67.27, 115.34, 119.70, 124.77, 126.77, 127.42, 128.05, 128.24, 128.29, 134.75, 136.17, 140.96, 143.39, 143.52, 155.51, 169.07, 172.03.

HRMS (M+H) Found 500.2078 (Calcd for C₃₀H₃₀NO₆ 500.2073).

6-(3,4,5-Tris-benzyloxy-6-benzyloxymethyl-tetrahydro-pyran-2-yl)-hex-4-enoic acid 2-benzyloxycarbonyl-2-(9H-fluoren-9-ylmethoxycarbonylamino)-ethyl ester (2.116) O-Pentenoyl serine **2.115** (60 mg, 0.12 mmol, 1 equiv) was reacted with **1.51b** (136 mg, 0.24 mmol, 2 equiv) and Grubbs second generation catalyst **1.17** (20 mg, 0.02 mmol, 0.2 equiv) according to General Procedure X. The crude product was purified by chromatography on silica (ethyl acetate/DCM, 1:19) and then recrystallised (pet ether/ethyl acetate) to give a mixture (42mg, 36%) of **2.116** and the double bond migrated product **2.116a** as a white solid (12:1 by ¹H NMR).

mp = 75-77°C

¹H NMR (CDCl₃, 500 MHz) from mixture **2.116** δ 2.24 (m, 4H, CH₂CH=CHCH₂), 2.42 (m, 2H, COCH₂), 3.59-3.76 (m, 6H, sugar moiety), 4.06 (m, 1H, H-C_{anomeric}), 4.22 (t, *J* = 6.7Hz, 1H, CH_{Fmoc}), 4.35-4.90 (m, 13H, CH₂Ph_{sugar}, CH_{2Fmoc}, NHCH, CH₂OCO), 5.19 (m, 2H, CH₂Ph_{benzyl ester}), 5.45 (m, 2H, HC=CH), 5.62 (d, *J* = 8.0Hz, 1H, NH), 7.11-7.75 (m, 33H, ArH).

Selected data ¹H NMR (CDCl₃, 500 MHz) from mixture **2.116a** δ 2.93 (d, *J* = 8.4 Hz, COCH₂CH=), 5.54 (m, CH=CH), 5.82 (m, NH).

^{13}C NMR (CDCl_3 , 75 MHz) from mixture **2.116** δ 27.65, 28.38, 33.59, 47.02, 53.478, 63.87, 67.26, 67.62, 68.86, 71.02, 72.96, 73.36, 73.84, 75.01, 75.35, 78.05, 80.00, 82.32, 119.95, 125.04, 127.05, 127.42, 127.52, 127.57, 127.70, 127.79, 127.86, 127.91, 128.27, 128.33, 128.36, 128.59, 130.18, 134.92, 137.98, 138.11, 138.16, 138.68, 141.23, 143.61, 155.69, 169.28, 172.45.

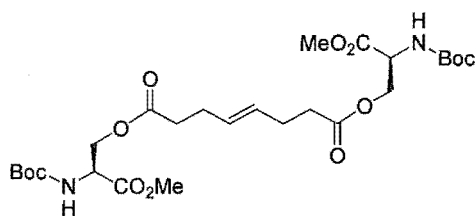
HRMS (M+H) Found 1036.4642 (Calcd for $\text{C}_{65}\text{H}_{66}\text{NO}_{16}$ 1036.4636).

Attempted synthesis of 6-(3,4,5-trihydroxy-6-hydroxymethyl-tetrahydro-pyran-2-yl)-hexanoic acid 2-carboxy-2-(9H-fluoren-9-ylmethoxycarbonylamino)-ethyl ester (2.117)

Compound **2.116** (40 mg, 0.02 mmol) was dissolved in methanol/ethyl acetate (20 mL, 1:1) and 10 % palladium/carbon (10 mg) was added. The mixture was stirred under an atmosphere of hydrogen (H_2 balloon) at rt overnight. The solution was filtered through celite to remove the catalyst, and the resulting organic solvent was removed by evaporation under reduced pressure to give a complex mixture of products (15 mg) as a yellow oil.

5.3 EXPERIMENTAL WORK AS DESCRIBED IN CHAPTER THREE

(2*S*)-Oct-4-enedioic acid bis-(2-*tert*-butoxycarbonylamino-2-methoxycarbonyl-ethyl) ester (**3.22**)



3.22

O-Pentenoyl serine **2.45** (50 mg, 0.17 mmol, 2 equiv) was treated with Grubbs second generation catalyst **1.17** (14 mg, 0.02 mmol, 0.2 equiv) according to General Procedure IX. The crude product was purified by flash chromatography on silica (ethyl acetate/DCM, 1:9) to give a mixture (42 mg, 86%) of **3.22** and the double bond migrated products **3.22a** and **b** as a brown oil (20:3:1 by ^1H NMR).

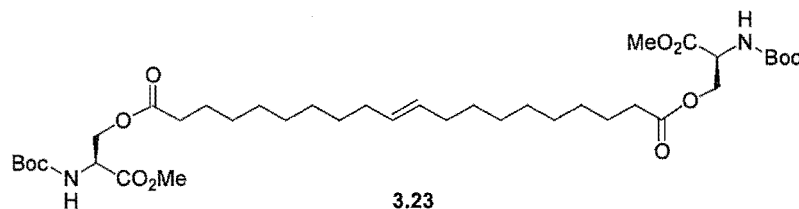
^1H NMR (CDCl_3 , 500 MHz) from mixture **3.22** δ 1.39 (s, 18H, $\text{C}(\text{CH}_3)_3$), 2.23 (m, 4H, $\text{CH}_2\text{CH}=\text{CH}_2$), 2.31 (m, 4H, COCH_2), 3.70 (s, 6H, OCH_3), 4.27 (m, 2H, OCH_2), 4.38 (m, 2H, OCH_2), 4.51 (m, 2H, NHCH), 5.32 (br m, 2H, NH), 5.38 (m, 2H, $\text{CH}=\text{CH}$).

Selected data ^1H NMR (CDCl_3 , 500 MHz) from mixture **3.22a** and **b** δ 3.01 (d, $J = 4.5$ Hz, $\text{COCH}_2\text{CH}=\text{}$), 3.09 (d, $J = 6.5$ Hz, $\text{COCH}_2\text{CH}=\text{}$), 5.52 (m, $\text{CH}=\text{CH}$).

^{13}C NMR (CDCl_3 , 75 MHz) from mixture **3.22** δ 27.44, 28.13, 33.61, 52.59, 52.74, 64.02, 80.15, 129.22, 155.02, 170.15, 172.36.

HRMS ($\text{M}+\text{H}$) Found 575.2818 (Calcd for $\text{C}_{26}\text{H}_{43}\text{N}_2\text{O}_{12}$ 575.2816).

(2*S*)-Icos-10-enedioic acid bis-(2-*tert*-butoxycarbonylamino-2-methoxycarbonyl-ethyl) ester (3.23)



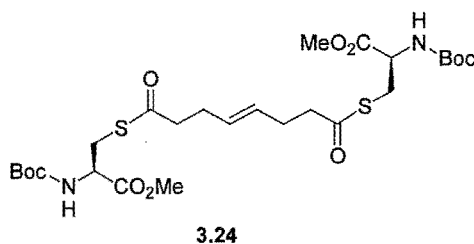
O-Decenoyl serine **2.46** (60 mg, 0.13 mmol, 2 equiv) was treated with Grubbs second generation catalyst **1.17** (14 mg, 0.02 mmol, 0.2 equiv) according to General Procedure IX. The crude product was purified by flash chromatography on silica (ethyl acetate/DCM, 1:9) to give **2.23** (30 mg, 62%) as a brown oil.

^1H NMR (CDCl_3 , 500 MHz) δ 1.28 (m, 18H, CH_2), 1.44 (s, 18H, $\text{C}(\text{CH}_3)_3$), 1.58 (m, 6H, CH_2), 1.96 (m, 4H, $=\text{CHCH}_2$), 2.28 (t, $J = 7.5$ Hz, 4H, COCH_2), 3.75 (s, 6H, OCH_3), 4.30 (m, 2H, OCH_2), 4.44 (m, 2H, OCH_2), 4.55 (m, 2H, NHCH), 5.27 (br d, $J = 8.0$ Hz, 2H, NH), 5.36 (m, 2H, $\text{CH}=\text{CH}$).

^{13}C NMR (CDCl_3 , 75 MHz) δ 24.68, 28.15, 28.93, 29.08, 29.17, 29.43, 29.47, 32.44, 33.84, 52.56, 52.81, 63.89, 80.13, 130.17, 155.01, 170.19, 173.16.

HRMS ($\text{M}+\text{H}$) Found 743.4714 (Calcd for $\text{C}_{38}\text{H}_{67}\text{N}_2\text{O}_{12}$ 743.4694).

(2*R*, 2*R*)-2-*tert*-Butoxycarbonylamino-3-[7-(2-*tert*-butoxycarbonylamino-2-methoxycarbonyl-ethylsulfanylcarbonyl)-hept-4-enoylsulfanyl]-propionic acid methyl ester (3.24)



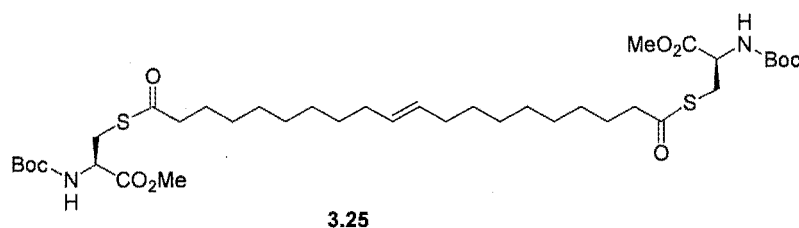
S-Pentenoyl cysteine **2.49a** (30 mg, 0.09 mmol, 2 equiv) was treated with Grubbs second generation catalyst **1.17** (8 mg, 0.01 mmol, 0.2 equiv) according to General Procedure IX. The crude product was purified by flash chromatography on silica (ethyl acetate/petroleum ether, 3:7) to give **3.24** (13 mg, 45%) as a brown oil.

^1H NMR (CDCl_3 , 500 MHz) δ 1.42 (s, 18H, $\text{C}(\text{CH}_3)_3$), 2.31 (m, 4H, $=\text{CHCH}_2$), 2.59 (t, $J = 7.4$ Hz, 4H, COCH_2), 3.32 (m, 4H, SCH_2), 3.72 (s, 6H, OCH_3), 4.50 (m, 2H, NHCH), 5.23 (br d, $J = 7.4$ Hz, 2H, NH), 5.41 (m, 2H, $\text{CH}=\text{CH}$).

^{13}C NMR (CDCl_3 , 75 MHz) δ 24.59, 28.23, 30.97, 43.50, 52.97, 52.61, 80.13, 129.14, 155.00, 170.89, 197.57.

HRMS ($\text{M}+\text{H}$) Found 607.2354 (Calcd for $\text{C}_{26}\text{H}_{43}\text{N}_2\text{O}_{10}\text{S}_2$ 607.2359).

(2*R*, 2*R*)-2-*tert*-Butoxycarbonylamino-3-[19-(2-*tert*-butoxycarbonylamino-2-methoxycarbonyl-ethylsulfanylcarbonyl)-nonadec-10-enoylsulfanyl]-propionic acid methyl ester (3.25)



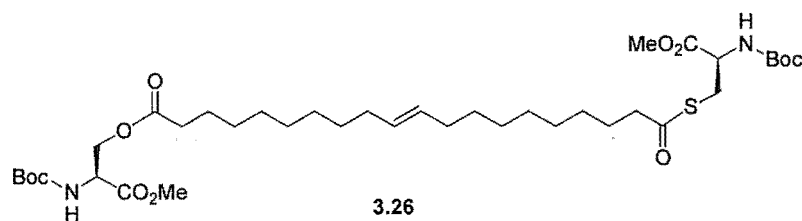
S-Decenoyl cysteine **2.48** (50 mg, 0.12 mmol, 2 equiv) was treated with Grubbs second generation catalyst **1.17** (10 mg, 0.01 mmol, 0.2 equiv) according to General Procedure IX. The crude product was purified by flash chromatography on silica (ethyl acetate/petroleum ether, 3:7) to give **3.25** (35 mg, 73%) as a brown oil.

^1H NMR (CDCl_3 , 500 MHz) δ 1.24 (m, 20H, CH_2), 1.41 (s, 18H, $(\text{CH}_3)_3$), 1.60 (m, 4H, COCH_2CH_2), 1.92 (m, 4H, $\text{CH}_2\text{CH}=\text{CH}_2$), 2.52 (m, 4H, COCH_2), 3.30 (m br, 4H, SCH_2), 3.71 (s, 6H, OCH_3), 4.49 (m br, 2H, NHCH), 5.22 (d br, $J = 7.9$ Hz, 2H, NH), 5.34 (m, 2H, $\text{CH}=\text{CH}$).

^{13}C NMR (CDCl_3 , 75 MHz) δ 25.48, 28.16, 28.74, 28.95, 29.07, 29.12, 29.46, 30.82, 32.44, 43.88, 52.51, 52.99, 79.99, 130.19, 154.95, 170.89, 198.39.

HRMS ($\text{M}+\text{H}$) Found 775.4224 (Calcd for $\text{C}_{38}\text{H}_{67}\text{N}_2\text{O}_{10}\text{S}_2$ 775.4237).

(2*R*, 2*S*)-19-(2-*tert*-Butoxycarbonylamino-2-methoxycarbonyl-ethylsulfanylcarbonyl)-nonadec-10-enoic acid-(2-*tert*-butoxycarbonylamino-2-methoxycarbonyl)-ethyl ester (3.26)



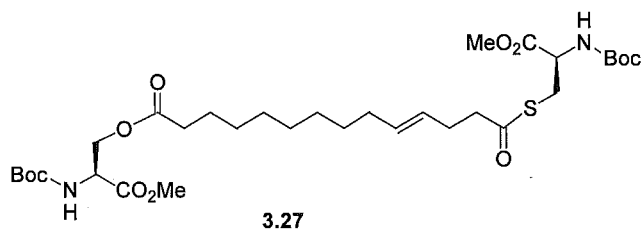
O-Decenoyl serine **2.46** (20 mg, 0.05 mmol, 1 equiv) and *S*-decenoyl cysteine **2.48** (42 mg, 0.10 mmol, 2 equiv) were treated with Grubbs second generation catalyst **1.17** (9 mg, 0.01 mmol, 0.2 equiv) according to General Procedure X. The crude product was purified by flash chromatography on silica (ethyl acetate/petroleum ether, 3:7) to give **3.26** (26 mg, 66%) as a brown oil.

^1H NMR (CDCl_3 , 500 MHz) δ 1.26 (m, 20H, CH_2), 1.41 (s, 9H, $\text{C}(\text{CH}_3)_3$), 1.43 (s, 9H, $\text{C}(\text{CH}_3)_3$), 1.59 (m, 4H, COCH_2CH_2), 1.93 (m, 4H, $\text{CH}_2\text{CH}=\text{CH}_2$), 2.27 (t, $J = 7.6$ Hz, 2H, OCOCH_2), 2.53 (t, $J = 7.5$ Hz, 2H, SCOCH_2), 3.31 (br m, 2H, SCH_2), 3.72 (s, 3H, OCH_3), 3.74 (s, 3H, OCH_3), 4.29 (m, 1H, OCHH), 4.42 (m, 1H, OCHH), 4.50 (m, 1H, NHCH), 4.54 (m, 1H, NHCH), 5.23 (br d, $J = 7.7$ Hz, 1H, NH), 5.27 (br d, $J = 8.3$ Hz, NH), 5.35 (m, 2H, $\text{CH}=\text{CH}$).

^{13}C NMR (CDCl_3 , 75 MHz) δ 24.74, 25.53, 28.21, 28.78, 28.81, 28.89, 29.00, 29.04, 29.12, 29.14, 29.18, 29.24, 29.53, 30.89, 32.51, 33.91, 40.28, 43.94, 52.58, 52.64, 52.87, 53.04, 63.98, 80.08, 80.25, 130.24, 130.29, 155.00, 155.08, 170.26, 170.94, 173.25, 198.46.

HRMS ($\text{M}+\text{H}$) Found 759.4453 (Calcd for $\text{C}_{38}\text{H}_{67}\text{N}_2\text{O}_{11}\text{S}$ 759.4466).

(2*R*, 2*S*)-13-(2-*tert*-Butoxycarbonylamino-2-methoxycarbonyl-ethylsulfanylcarbonyl)-tridec-10-enoic acid-(2-*tert*-butoxycarbonylamino-2-methoxycarbonyl)-ethyl ester (3.27)



O-Decenoyl serine **2.46** (20 mg, 0.05 mmol, 1 equiv) and *S*-pentenoyl cysteine **2.49a** (33 mg, 0.1 mmol, 2 equiv) were treated with Grubbs second generation catalyst **1.17** (9 mg, 0.01 mmol, 0.2 equiv) according to General Procedure X. The crude product was purified by flash chromatography on silica (ethyl acetate/petroleum ether, 2:3) to give **3.27** (18 mg, 51%) as a brown oil.

^1H NMR (CDCl_3 , 500 MHz) δ 1.24 (m, 10H, CH_2), 1.42 (s, 9H, $\text{C}(\text{CH}_3)_3$), 1.43 (s, 9H, $\text{C}(\text{CH}_3)_3$), 1.56 (m, 2H, CH_2), 1.94 (m, 2H, $=\text{CHCH}_2(\text{CH}_2)_7\text{CO}$), 2.29 (m, 4H, OCOCH_2 , $=\text{CHCH}_2\text{CH}_2\text{CO}$), 2.59 (t, $J = 7.5$ Hz, 2H, SCOCH_2), 3.32 (br m, 2H, CH_2S), 3.72 (s, 3H, OCH_3), 3.74 (s, 3H, OCH_3), 4.29 (m, 1H, OCHH), 4.43 (m, 1H, OCHH), 4.50 (m, 1H, NHCH), 4.54 (m, 1H, NHCH), 5.22 (br d, $J = 7.4$ Hz, 1H, NH), 5.27 (br d, $J = 8.5$ Hz, 1H, NH), 5.33 (dt, $J = 6.7, 15.2$ Hz, 1H, $\text{CH}=\text{CH}$), 5.42 (dt, $J = 6.6, 15.2$ Hz, 1H, $\text{CH}=\text{CH}$).

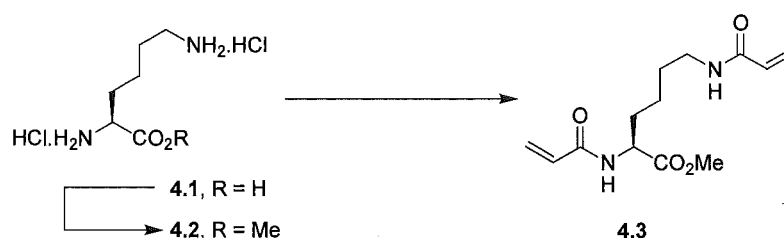
^{13}C NMR (CDCl_3 , 75 MHz) δ 24.72, 27.10, 28.20, 28.38, 28.97, 29.11, 29.19, 29.25, 30.89, 32.36, 33.89, 43.87, 52.57, 52.62, 52.86, 53.00, 63.97, 80.07, 80.22, 127.10, 132.23, 154.98, 155.05, 170.25, 170.89, 173.22, 197.76.

HRMS ($\text{M}+\text{H}$) Found 675.3527 (Calcd for $\text{C}_{32}\text{H}_{55}\text{N}_2\text{O}_{11}\text{S}$ 675.3527).

5.4 EXPERIMENTAL WORK AS DESCRIBED IN CHAPTER FOUR

5.4.1 Lysine-based single amino acid cyclic compounds

Preparation of (2*S*)-2,6-bis-acryloylamino-hexanoic acid methyl ester (4.3)



(2*S*)-2,6-Diamino-hexanoic acid methyl ester dihydrochloride salt (4.2) *L*-Lys.HCl **4.1** (3 g, 16.5 mmol) was suspended in 2,2-dimethoxypropane (200 mL) and concentrated hydrochloric acid (16.5 mL) was added. Methanol (60 mL) was added and the solution was refluxed for 2 h, and then stirred at rt for 18 h. The solvents were removed by evaporation under reduced pressure and the residue dissolved in a minimum volume of methanol. Ether (300 mL) was added until the product crashed out of solution. The solid was filtered, and then recrystallised from water/acetone and 2,2-dimethoxypropane to give *L*-Lys-OMe.2HCl **4.2** (3.26 g, 84%) as a white solid.

mp = 205-207°C. NMR data was consistent with the literature.¹⁷

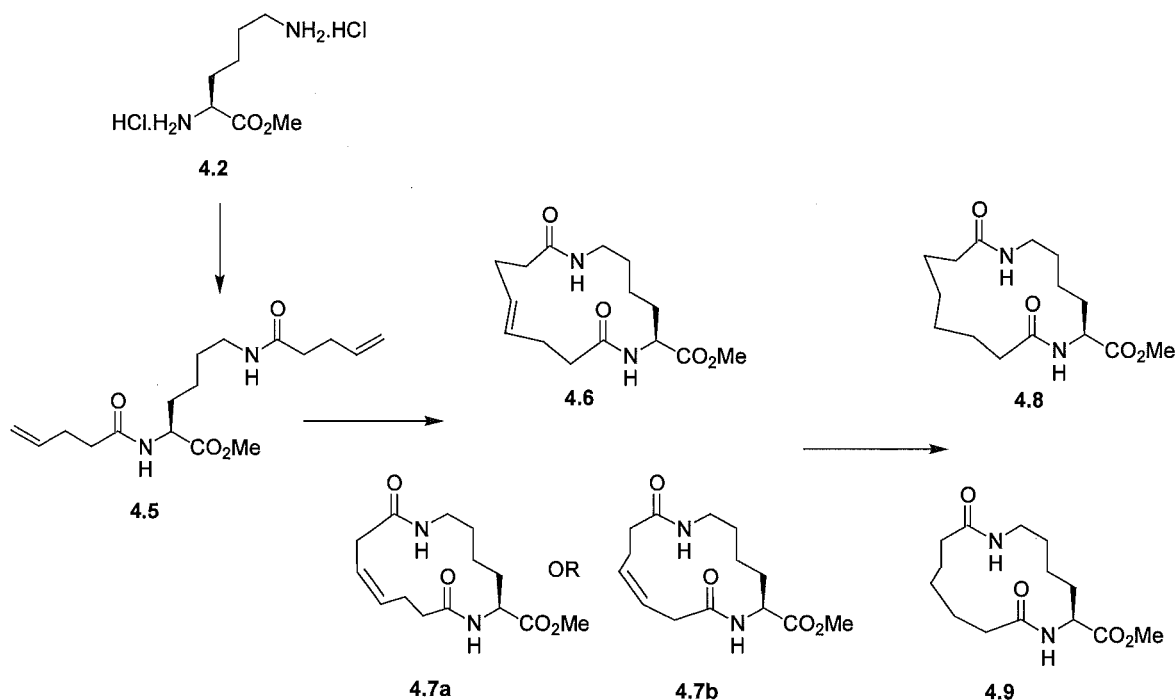
(2*S*)-2,6-Bis-acryloylamino-hexanoic acid methyl ester (4.3) To a stirred solution of *L*-Lys-OMe.2HCl **4.2** (50 mg, 0.12 mmol, 1 equiv) in DCM (2.5 mL) was added DIPEA dropwise at 0°C until a pH of 8-9 (Universal indicator paper) was obtained. Acryloyl chloride (69 mg, 0.77 mmol, 3.6 equiv) was added dropwise and the reaction warmed to rt with stirring overnight. The solution was washed successively three times with 1 M aqueous hydrochloric acid and water. The organic layer was dried (Na₂SO₄), and the solvent was removed by evaporation under reduced pressure. The crude product was purified by flash chromatography on silica (methanol/ethyl acetate, 1:19) to give **4.3** (8 mg, 14%) as a sticky, yellow solid.

mp = >340°C, decomposed

^1H NMR (CDCl_3 , 500 MHz) δ 1.32 (m, 2H, CHCH_2CH_2), 1.50 (m, 2H, $\text{CH}_2\text{CH}_2\text{NH}$), 1.71 (m, 1H, NHCHCHH), 1.80 (m, 1H, NHCHCHH), 3.24 (m, 2H, CH_2NH), 3.67 (s, 3H, OCH_3), 4.53 (m, 1H, CH), 5.58 (m, 2H, $\text{CH}=\text{CH}_2$), 6.11-6.23 (m, 4H, $\text{CH}=\text{CH}_2$), 6.60 (br s, 1H, CH_2NH), 6.93 (d, 1H, $J = 7.3$ Hz, NHCH).

^{13}C NMR (CDCl_3 , 75 MHz) δ 22.19, 28.56, 31.33, 38.50, 51.91, 52.28, 126.02, 126.97, 130.26, 130.87, 165.64, 166.04, 172.73.

Preparation of (2S)-8,15-dioxo-1,7-diaza-cyclopentadec-11-ene-2-carboxylic acid methyl ester (4.6) and (2S)-8,15-dioxo-1,7-diaza-cyclopentadecane-2-carboxylic acid methyl ester (4.8)



(2S)-2,6-Bis-pent-4-enoylamino-hexanoic acid methyl ester (4.5) To a stirred solution of *L*-Lys-OMe·2HCl **4.2** (1 g, 4.3 mmol, 1.1 equiv) and 4-pentenoic acid (774 mg, 7.7 mmol, 2 equiv) in DCM (60 mL) was added EDCI (1.93 g, 10.1 mmol, 2.6 equiv) and HOBt (1.57 g, 11.6 mmol, 3 equiv). DIPEA (1.60 mL, 8.47 mmol, 2.2 equiv) was added and the solution stirred overnight. The mixture was diluted with DCM (60 mL), and washed twice with saturated brine (100 mL). The aqueous washings were combined and back extracted twice with DCM (100 mL). The organic washings were combined, dried (MgSO_4), and the solvent was removed by evaporation under reduced pressure. The crude product was

purified by flash chromatography on silica (DCM/ethyl acetate, 1:9) to give **4.5** (1.02 g, 74%) as a waxy, pale yellow solid.

mp = 47-49°C

^1H NMR (CDCl_3 , 500 MHz) δ 1.30 (m, 2H, CHCH_2CH_2), 1.48 (m, 2H, $\text{CH}_2\text{CH}_2\text{NH}$), 1.66 (m, 1H, NHCHCHH), 1.78 (m, 1H, NHCHCHH), 2.22-2.36 (m, 8H, COCH_2CH_2), 3.19 (m, 2H, CH_2NH), 3.69 (s, 3H, OCH_3), 4.51 (m, 1H, NHCH), 4.99 (m, 4H, $\text{CH}=\text{CH}_2$), 5.77 (m, 2H, $\text{CH}=\text{CH}_2$), 6.02 (br s, 1H, CH_2NH), 6.42 (d, $J = 7.3$ Hz, 1H, CHNH).

^{13}C NMR (CDCl_3 , 75 MHz) δ 22.04, 28.41, 28.99, 29.18, 30.64, 34.58, 34.96, 38.08, 51.54, 51.60, 114.66, 114.72, 136.41, 136.54, 172.32, 172.40, 172.49.

HRMS ($\text{M}+\text{H}$) Found 325.2130 (Calcd for $\text{C}_{17}\text{H}_{29}\text{N}_2\text{O}_4$ 325.2127).

(2S)-8,15-Dioxo-1,7diazacyclopentadec-11-ene-2-carboxylic acid methyl ester (4.6) and (2S)-8,14-dioxo-1,7diazacyclotetradec-11-ene-2-carboxylic acid methyl ester (4.7a/b)

Diene **4.5** (200 mg, 0.62 mmol, 1 equiv) was treated with Grubbs second generation catalyst **1.17** (105 mg, 0.02 mmol, 0.2 equiv) according to General Procedure I. The crude product was purified by chromatography on silica (methanol/ethyl acetate, 1:9) to give an isomer mixture (134 mg, 73%) as a brown solid. The product was isolated as a mixture of 15-membered **4.6** and ring-contracted 14-membered **4.7a/b** products in a 3:1 ratio respectively, that were inseparable on silica-gel based column chromatography. NB. There are two possible 14-membered ring-contracted products, which one of these that formed was not determined.

mp = 200-203°C (isomer mixture)

^1H NMR (CDCl_3 , 500 MHz) from mixture **4.6** δ 1.23-1.90 (m, 6H, $\text{NHCH}(\text{CH}_2)_3$), 2.16-2.52 (m, 8H, COCH_2CH_2), 2.98 (m, 1H, NHCHH), 3.55 (m, 1H, NHCHH), 3.71 (s, 3H, OCH_3), 4.59 (m, 1H, NHCH), 5.51 (m, 2H, $\text{CH}=\text{CH}$), 5.76 (br s, 1H, CH_2NH), 6.20 (br d, $J = 7.5$ Hz, 1H, CHNH).

Selected data ^1H NMR (CDCl_3 , 500 MHz) from mixture **4.7a/b** δ 3.05 (m, NHCHH), 3.43 (m, NHCHH), 3.73 (s, OCH_3), 4.46 (m, NHCH), 5.33 (m, $\text{CH}=\text{CH}$), 6.05 (br s, CH_2NH), 6.48 (br d, $J = 5.5$ Hz, CHNH).

^{13}C NMR (CDCl_3 , 75 MHz) from mixture **4.6** δ 21.74, 28.02, 28.13, 28.41, 32.06, 36.15, 36.42, 37.94, 51.15, 52.38, 129.84, 129.97, 172.39, 172.88, 173.11.

Selected data ^{13}C NMR (CDCl_3 , 75 MHz) from mixture **4.7a/b** δ 21.07, 26.69, 26.85, 31.37, 35.60, 35.65, 37.67, 51.19, 52.32, 129.69, 172.83, 173.03.

Mass spectrometry showed both 15-membered **4.6** ($M+H = 297$) and ring contracted 14-membered **4.7a/b** ($M+H = 283$) compounds were present.

(2S)-8,15-Dioxo-1,7diazacyclopentadecane-2-carboxylic acid methyl ester (4.8) and (2S)-8,14-dioxo-1,7diazacyclotetradecane-2-carboxylic acid methyl ester (4.9) The isomer mixture of **4.6** and **4.7a/b** (51 mg, 0.17 mmol, 1 equiv) was treated with 10% palladium/carbon (10 mg) according to General Procedure III. This gave a mixture of 15-membered **4.8** and ring-contracted 14-membered **4.9** (51 mg, 100%) products in a 3:1 ratio respectively as a pink solid.

mp = 209–211°C (mixture)

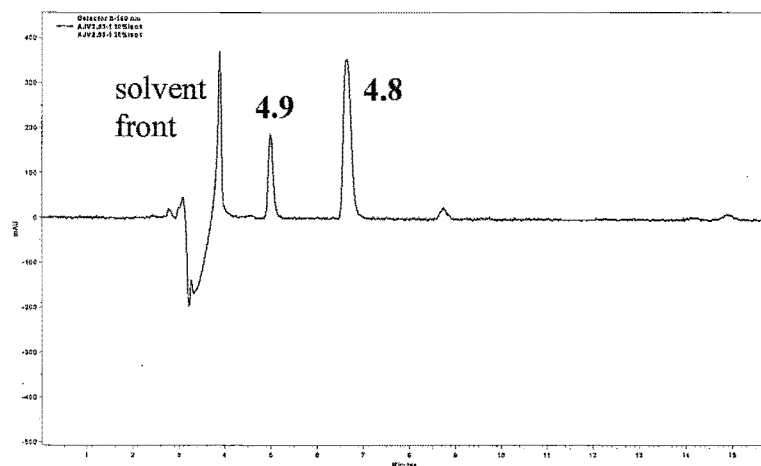
^1H NMR (CDCl_3 , 500 MHz) from mixture **4.8** δ 1.23–1.72 (m, 6H, $\text{NHCH}(\text{CH}_2)_3$), 2.06–2.36 (m, 8H, COCH_2CH_2), 2.91 (m, 1H, NHCHH), 3.64 (m, 1H, NHCHH), 3.72 (s, 3H, OCH_3), 4.62 (m, 1H, NHCH), 5.67 (br s, 1H, CH_2NH), 6.17 (br d, $J = 8.4$ Hz, 1H, CHNH).

Selected data ^1H NMR (CDCl_3 , 500 MHz) from mixture **4.9** δ 2.99 (m, NHCHH), 4.67 (m, NHCH), 5.86 (br s, CH_2NH), 6.35 (br d, $J = 8.9$ Hz, CHNH).

^{13}C NMR (CDCl_3 , 75 MHz) from mixture **4.8** δ 21.98, 24.78, 26.18, 26.73, 27.76, 28.68, 32.11, 35.69, 35.84, 37.95, 51.20, 52.38, 173.06, 173.23, 173.59.

Selected data ^{13}C NMR (CDCl_3 , 75 MHz) from mixture **4.9** δ 21.27, 24.71, 26.22, 26.88, 27.49, 31.04, 34.73, 35.54, 37.38, 50.90, 52.35, 173.11, 173.40, 173.72.

HPLC trace of **4.8** and **4.9** isomer mixture in 20% acetonitrile/water + 0.05% TFA.



Isomer mixture **4.6/4.7a/b** was then examined by analytical HPLC. A sample (1 mg/mL in MeOH) was run in an isocratic solvent system of 20% acetonitrile/water with 0.05% TFA. The trace showed two peaks at different retention times in a 3:1 ratio. Semi-preparative HPLC was carried out in an isocratic system of 20% acetonitrile/water with 0.05% TFA to obtain pure samples of individual isomers. Refer to General Procedures section **5.1** for details of the reverse columns used. One purified both **4.8** and **4.9** were only sparingly soluble in CDCl_3 . ^1H NMR spectra were run in CDCl_3 to observe NH peaks, but experimental data given below is in CD_3OD as compounds were more soluble in this.

^1H NMR (CD_3OD , 500 MHz) **4.8** δ 1.33 (m, 8H, CH_2), 1.53 (m, 6H, CH_2), 1.65 (m, 1H, NHCHCHH), 1.78 (m, 3H, NHCHH , CH_2), 2.25 (m, 4H, COCH_2), 3.01 (m, 1H, NHCHH), 3.41 (m, 1H, NHCHH), 3.69 (s, 3H, OCH_3), 4.45 (m, 1H, CH).

^{13}C NMR (CD_3OD , 75 MHz) **4.8** δ 24.27, 26.51, 26.80, 29.50, 29.57, 30.12, 32.53, 36.31, 36.74, 39.68, 52.90, 53.03, 174.53, 174.58, 176.56.

HRMS (M+H) Found 299.1978 (Calcd for $\text{C}_{15}\text{H}_{27}\text{N}_2\text{O}_4$ 299.1972).

^1H NMR (CD_3OD , 500 MHz) **4.9** δ 1.33 (m, 6H, CH_2), 1.60 (m, 5H, NHCHCHH , CH_2), 1.88 (m, 1H, NHCHCHH), 2.22 (m, 4H, COCH_2), 3.07 (m, 1H, NHCHH), 3.40 (m, 1H, NHCHH), 3.70 (s, 3H, OCH_3), 4.50 (m, 1H, CH).

^{13}C NMR (CD_3OD , 75 MHz) **4.9** δ 22.20, 25.17, 25.28, 27.03, 27.92, 30.45, 35.38, 35.57, 37.91, 51.00, 51.39, 172.85, 174.74, 174.75.

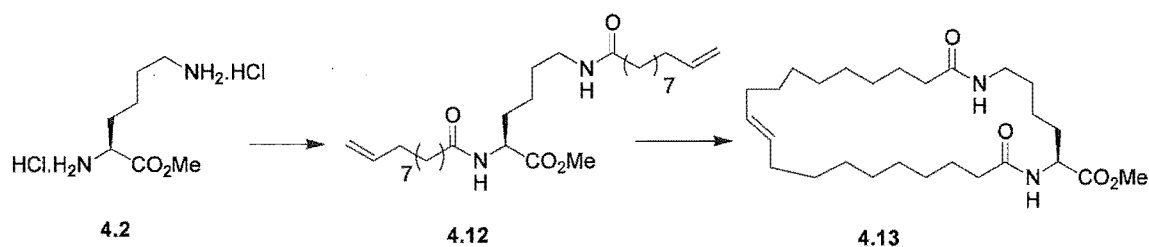
HRMS (M+H) Found 285.1811 (Calcd for $\text{C}_{14}\text{H}_{25}\text{N}_2\text{O}_4$ 285.1814).

Synthesis of **4.6/4.7a/b** under microwave conditions

Diene **4.5** (70 mg, 0.22 mmol, 1 equiv) was dissolved in TCE (20 mL) in a 100 mL glass round-bottomed flask. Grubbs second generation catalyst **1.17** (13 mg, 0.02 mmol, 0.7 equiv) was added, the flask connected to a reflux condenser in the microwave, and the solution heated at 800 W for 3 min. The addition of catalyst (2×13 mg) followed by heating (800 W, 3 min) was repeated twice more. The solvent was removed by evaporation under reduced pressure to give the crude product. The crude product was purified by chromatography on silica (methanol/ethyl acetate, 1:9) to give an isomer mixture (45mg, 70%) as a brown solid. This product was isolated as a mixture of **4.6** and

4.7a/b in a 4:1 ratio respectively. NMR data of the product mixture was consistent with that of refluxing DCM conditions.

Preparation of (2*S*)-8,27-dioxo-1,7-diaza-cycloheptacos-17-ene-2-carboxylic acid methyl ester (4.13)



(2*S*)-2,6-Bis-undec-10-enoylamino-hexanoic acid methyl ester (4.12) To a stirred solution of *L*-Lys-OMe.2HCl **4.2** (500 mg, 2.2 mmol, 1 equiv) and 10-decenoic acid (920 mg, 5.06 mmol, 2.3 equiv) in DCM (80 mL) was added EDCI (1.09 g, 5.72 mmol, 2.6 equiv) and HOBt (891 mg, 6.6 mmol, 3 equiv). DIPEA (0.93 mL, 5.06 mmol, 2.3 equiv) was added and the solution stirred overnight. The reaction was worked-up as described in General Procedure VII. The crude product was purified by flash chromatography on silica (petroleum ether/ethyl acetate, 3:7) to give **4.12** (980 mg, 92%) as a white solid.

mp = 61–63°C

^1H NMR (CDCl_3 , 500 MHz) δ 1.23–1.68 (m, 30H, CH_2), 2.01 (m, 4H, $\text{CH}_2\text{CH}=\text{CH}_2$), 2.16 (m, 2H, COCH_2), 2.22 (m, 2H, COCH_2), 3.22 (m, 2H, CH_2NH), 3.72 (s, 3H, OCH_3), 4.55 (m, 1H, NHCH), 4.91 (m, 2H, $\text{CH}=\text{CHH}$), 4.97 (ddd, $J = 1.6, 3.5, 17.2$ Hz, 2H, $\text{CH}=\text{CHH}$), 5.57 (br s, 1H, CH_2NH), 5.78 (m, 2H, $\text{CH}=\text{CH}_2$), 6.17 (d, $J = 7.3$ Hz, 1H, NHCH).

^{13}C NMR (CDCl_3 , 75 MHz) δ 21.98, 25.21, 25.39, 28.34, 28.54, 28.76, 28.85, 30.57, 33.22, 35.58, 35.97, 37.89, 51.49, 51.58, 113.64, 138.35, 172.39, 173.22, 173.35.

HRMS ($\text{M}+\text{H}$) Found 493.3992 (Calcd for $\text{C}_{29}\text{H}_{53}\text{N}_2\text{O}_4$ 493.4005).

(2S)-(8,27-Dioxo-1,7-diaza-cycloheptacos-17-ene-2-carboxylic acid methyl ester (4.13)

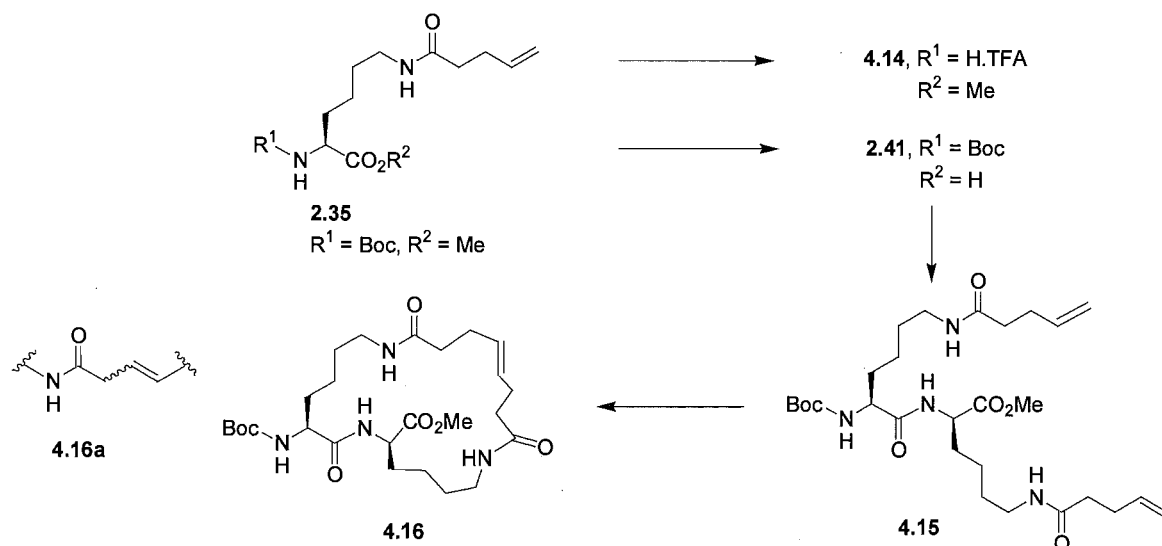
Diene **4.12** (150 mg, 0.31 mmol, 1 equiv) was treated with Grubbs second generation catalyst **1.17** (52 mg, 0.06 mmol, 0.2 equiv) according to General Procedure I. The crude product was purified twice by chromatography on silica (methanol/DCM, 1:19 then methanol/ethyl acetate 1:19) to give **4.13** (28 mg, 20%) as a brown solid.

mp = 93-94°C

^1H NMR (CDCl_3 , 500 MHz) δ 1.29 (m, 22H, CH_2), 1.63 (m, 7H, CH_2 , NHCHCHH), 1.81 (m, 1H, NHCHCHH), 1.99 (m, 4H, $=\text{CHCH}_2$), 2.20 (m, 4H, COCH_2), 3.17 (m, 1H, NHCHH), 3.33 (m, 1H, NHCHH), 3.72 (s, 3H, OCH_3), 4.53 (m, 1H, NHCH), 5.31 (m, 2H, $\text{CH}=\text{CH}$), 5.84 (br m, 1H, NH), 6.38 (br m, 1H, NH).

^{13}C NMR (CDCl_3 , 75 MHz) δ 21.68, 25.67, 25.88, 28.26, 28.50, 28.84, 28.88, 29.05, 29.13, 29.22, 29.30, 29.39, 29.44, 31.87, 31.90, 31.97, 32.02, 36.49, 36.52, 36.78, 51.40, 52.37, 130.71, 130.80, 173.08, 173.68, 173.90.

HRMS ($\text{M}+\text{H}$) Found 465.3700 (Calcd for $\text{C}_{27}\text{H}_{49}\text{N}_2\text{O}_4$ 465.3692).

5.4.2 Lysine-based dipeptide cyclic compounds**Preparation of (6S, 9S)-9-tert-butoxycarbonylamino-8,15,22-trioxo-1,7,14-triazacyclodocos-18-ene-6-carboxylic acid methyl ester (4.16)**

(*2S*, *2S*)-2-[2-*tert*-Butoxycarbonylamino-6-(*pent*-4-enoylamino)-hexanoylamino]-6-(*pent*-4-enoylamino)-hexanoic acid methyl ester (**4.15**) *N*_ε-Pentenoyl lysine **2.35** (300 mg, 0.88 mmol) was Boc-protected using TFA according to General Procedure VI to give TFA salt **4.14**. In a separate reaction **2.35** (300 mg, 0.88 mmol) was treated with 0.2 M aqueous lithium hydroxide (2 equiv) to give the free carboxylic acid **2.41** according to General Procedure VIII. The TFA salt **4.14** (assume 0.88 mmol) and the carboxylic acid **2.41** (assume 0.88 mmol) were treated with HATU (401 mg, 1.06 mmol, 1.2 equiv) according to General Procedure V. The crude product was purified by flash chromatography on silica (methanol/DCM, 1:19) to give **4.15** (260 mg, 54%) as a white solid.

mp = 104 – 107°C

¹H NMR (CDCl₃, 500 MHz) δ 1.28-1.53 (8H, CH₂), 1.41 (s, 9H, (CH₃)₃), 1.72 (m, 2H, NHCHCHH), 1.82 (m, 2H, NHCHCHH), 2.25 (m, 4H, COCH₂CH₂), 2.35 (m, 4H, COCH₂), 3.20 (m, 4H, CH₂NH), 3.69 (s, 3H, OCH₃), 4.12 (m, 1H, NHCH), 4.46 (m, 1H, NHCH), 4.99 (m, 4H, CH=CH₂), 5.39 (br d, *J* = 7.5 Hz, 1H, CHNH), 5.78 (m, 2H, CH=CH₂), 6.08 (m, 2H, CH₂NH), 7.04 (br d, *J* = 7.1 Hz, 1H, CHNH).

¹³C NMR (CDCl₃, 75 MHz) δ 22.03, 22.24, 27.99, 28.56, 28.64, 29.36, 29.39, 30.72, 31.72, 35.24, 38.26, 38.33, 51.88, 51.95, 53.88, 79.34, 114.94, 114.98, 136.80, 155.49, 172.34, 172.42, 172.52, 172.75.

HRMS (M+H) Found 553.3603 (Calcd for C₂₈H₄₉N₄O₇ 553.3601).

(*6S*, *9S*)-9-*tert*-Butoxycarbonylamino-8,15,22-trioxo-1,7,14-triaza-cyclodocos-18-ene-6-carboxylic acid methyl ester (**4.16**) Diene **4.15** (149 mg, 0.27 mmol, 1 equiv) was treated with Grubbs second generation catalyst **1.17** (46 mg, 0.05 mmol, 0.2 equiv) according to General Procedure I. The crude product was purified by chromatography on silica (methanol/DCM, 1:9) to give a mixture (42 mg, 30%) of **4.16** and double bond migrated product **4.16a** as a brown oil (9:1 by ¹H NMR).

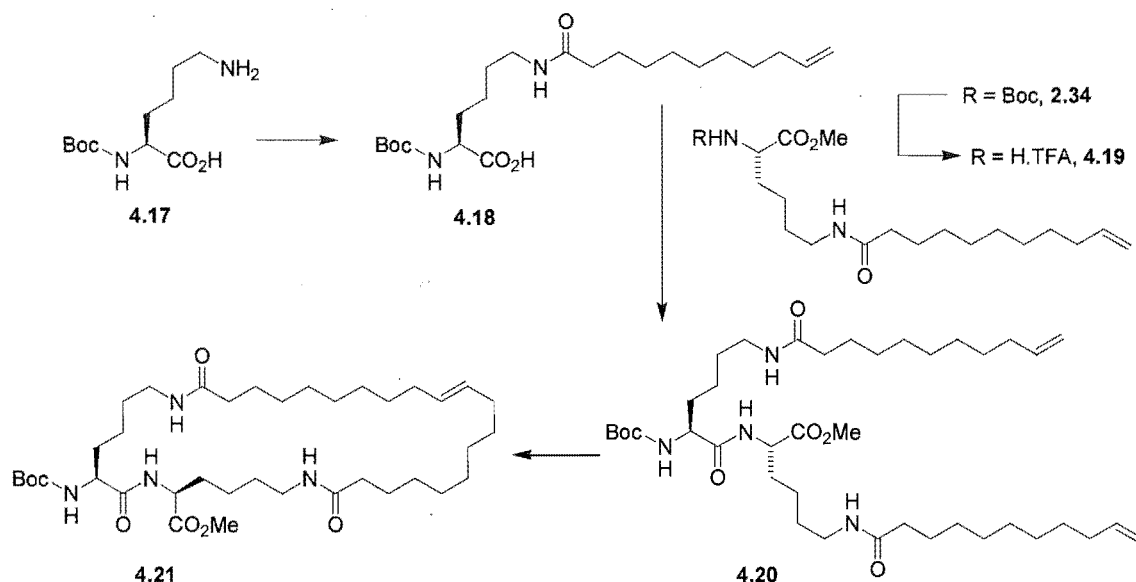
¹H NMR (CD₃OD, 500 MHz) from mixture **4.16** δ 1.28-1.50 (m, 8H, CH₂), 1.44 (s, 9H, C(CH₃)₃), 1.78 (m, 4H, CH₂), 2.25 (m, 4H, COCH₂CH₂), 3.15 (m, 4H, NHCH₂), 3.70 (s, 3H, OC H₃), 4.01 (m, 1H, NHCH), 4.37 (m, 1H, NHCH), 5.45 (m, 2H, CH=CH).

Selected data ¹H NMR (CD₃OD, 500 MHz) from mixture **4.16a** δ 2.88 (d, *J* = 5.0 Hz, NHCOCH₂CH=), 5.57 (m, CH=CH).

^{13}C NMR (CD_3OD , 75 MHz) from mixture **4.16** δ 23.56, 24.55, 29.01, 29.95, 30.07, 30.22, 30.76, 32.04, 33.59, 37.07, 37.42, 39.37, 40.24, 53.04, 53.52, 56.58, 80.89, 130.51, 131.40, 157.91, 174.09, 175.41, 175.76.

HRMS ($\text{M}+\text{H}$) Found 525.3301 (Calcd for $\text{C}_{26}\text{H}_{45}\text{N}_4\text{O}_7$ 525.3288).

Preparation of (6*S*, 9*S*)-9-*tert*-butoxycarbonylamino-8,15,34-trioxo-1,7,14-triazacyclotetratriciacont-24-ene-6-carboxylic acid methyl ester (4.21**)**



(2*S*)-2-*tert*-Butoxycarbonylamino-6-(undec-10-enoylamino)-hexanoic acid (4.18**)** Boc-*L*-Lysine **4.17** (2 g, 8.10 mmol, 1 equiv) was treated with 10-decenoyl chloride (1.92 mL, 8.91 mmol, 1.1 equiv) according to General Procedure IV. The crude product was purified by flash chromatography on silica (DCM/ethyl acetate, 1:1, then ethyl acetate 100%) to give **4.18** (2.36 g, 64%) as a white solid.

mp = 68 – 72°C

^1H NMR (CDCl_3 , 500 MHz) δ 1.26-1.61 (m, 16H, CH_2), 1.43 (s, 9H, $\text{C}(\text{CH}_3)_3$), 1.70 (m, 1H, NHCHCHH), 1.84 (m, 1H, NHCHCHH), 2.02 (m, 2H, $\text{CH}_2\text{CH}=\text{CH}_2$), 2.16 (t, $J = 7.7$ Hz, 2H, COCH_2), 3.23 (m, 2H, CH_2NH), 4.27 (m, 1H, NHCH), 4.92 (dd, $J = 1.2, 10.3$ Hz, 2H, $\text{CH}=\text{CHH}$), 4.97 (ddd, $J = 1.6, 3.5, 17.1$ Hz, 1H, $\text{CH}=\text{CHH}$), 5.28 (br d, $J = 7.9$ Hz, 1H, CHNH), 5.59 (m, 1H, $\text{CH}=\text{CH}_2$), 5.85 (br s, 1H, CH_2NH).

^{13}C NMR (CDCl_3 , 75 MHz) δ 22.21, 25.56, 27.99, 28.49, 28.69, 28.94, 28.97, 31.92, 33.41, 36.12, 38.96, 52.84, 79.39, 113.85, 138.65, 155.55, 174.34, 174.70.

HRMS ($\text{M}+\text{H}$) Found 413.3034 (Calcd for $\text{C}_{22}\text{H}_{41}\text{N}_2\text{O}_5$ 413.3015).

(2*S*, 2*S*)-2-[2-*tert*-Butoxycarbonylamino-6-(undec-10-enoylamino)-hexanoylamino]-6-(un-dec-10-enoylamino)-hexanoic acid methyl ester (4.20) N_{α} -Boc- N_{ϵ} -decenoyl-*L*-Lys-OH **2.34** (230 mg, 0.54 mmol) was Boc-deprotected using TFA (2 equiv) according to General Procedure VI to give **4.19**. The TFA salt **4.19** (0.54 mmol, 1 equiv) and the carboxylic acid **4.18** (223 mg, 0.54 mmol, 1 equiv) were treated with HATU (246 mg, 0.65 mmol, 1.2 equiv) according to General Procedure V. The crude product was purified by flash chromatography on silica (methanol/DCM, 1:19) to give **4.20** (340 mg, 87%) as a dirty white solid.

mp = 75 – 78°C

^1H NMR (CDCl_3 , 500 MHz) δ 1.26 (s, 9H, $\text{C}(\text{CH}_3)_3$), 1.26-1.61 (m, 32H, CH_2), 1.71 (m, 2H, CHNHCHH), 1.82 (m, 2H, CHNHCHH), 2.01 (m, 4H, $\text{CH}_2\text{CH}=\text{CH}_2$), 2.14 (m, 4H, COCH_2), 3.21 (m, 4H, CH_2NH), 3.71 (s, 3H, OCH_3), 4.11 (m, 1H, NHCH), 4.48 (m, 1H, NHCH), 4.90 (m, 2H, $\text{HC}=\text{CH}_2$), 4.96 (ddd, $J = 1.6, 3.3, 17.1$ Hz, 2H, $\text{CH}=\text{CH}_2$), 5.31 (br d, $J = 7.1$ Hz, 1H, CHNH), 5.78 (m, 2H, $\text{CH}=\text{CH}_2$), 5.86 (m, 2H, CH_2NH), 6.94 (d, $J = 7.5$ Hz, 1H, CHNH).

^{13}C NMR (CDCl_3 , 75 MHz) δ 22.08, 22.28, 23.12, 24.14, 25.58, 28.96, 28.03, 28.56, 28.76, 29.05, 30.75, 31.38, 31.86, 33.45, 36.28, 38.29, 38.46, 47.05, 51.96, 53.85, 79.37, 108.96, 113.86, 133.82, 138.73, 155.52, 167.51, 172.40, 173.54, 173.78.

HRMS ($\text{M}+\text{H}$) Found 721.5482 (Calcd for $\text{C}_{40}\text{H}_{73}\text{N}_4\text{O}_7$ 721.5479).

(6*S*),(9*S*)-9-*tert*-Butoxycarbonylamino-8,15,34-trioxo-1,7,14-triaza-cyclotettratriacont-24-ene-6-carboxylic acid methyl ester (4.21) Diene **4.20** (218 mg, 0.30 mmol, 1 equiv) was treated with Grubbs second generation catalyst **1.17** (51 mg, 0.06 mmol, 0.02 equiv) according to General Procedure I. The crude product was precipitated in ethyl acetate to give a brown solid that was purified by flash chromatography on silica (methanol/ethyl acetate, 1:19) to give **4.21** (59 mg, 28%) as a brown solid.

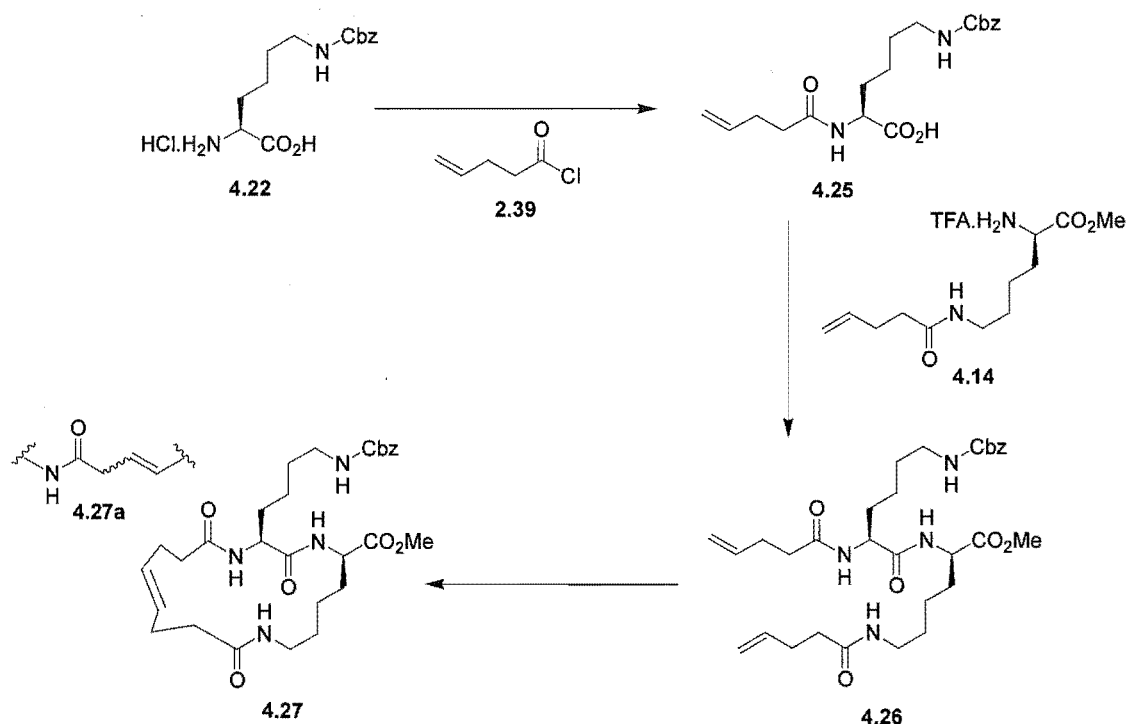
mp = 99 – 101°C

^1H NMR (CD_3OD , 500 MHz) δ 1.30 (m, 26H, CH_2), 1.43 (s, 9H, $\text{C}(\text{CH}_3)_3$), 1.51 (m, 2H, CH_2), 1.59 (m, 5H, CHNHCHH , CH_2), 1.72 (m, 2H, CHNHCHH), 1.84 (m, 1H, CHNHCHH), 1.99 (m, 4H, $\text{CH}_2\text{CH}=\text{CH}_2$), 2.16 (t, $J = 7.5$ Hz, 4H, COCH_2), 3.16 (m, 4H, NHCH_2), 3.70 (s, 3H, OCH_3), 4.03 (m, 1H, NHCH), 4.39 (m, 1H, NHCH), 5.38 (m, 2H, $\text{CH}=\text{CH}$).

^{13}C NMR (CD_3OD , 75 MHz) δ 18.10, 20.04, 21.45, 24.31, 24.39, 27.35, 29.08, 30.14, 30.18, 30.35, 30.48, 30.61, 30.74, 30.90, 31.03, 32.34, 33.27, 33.91, 37.45, 40.21, 40.32, 53.01, 53.73, 56.01, 80.70, 131.24, 131.74, 157.91, 174.14, 175.45, 175.52, 176.34.

HRMS ($\text{M}+\text{H}$) Found 693.5161 (Calcd for $\text{C}_{38}\text{H}_{69}\text{N}_4\text{O}_7$ 693.5166).

Preparation of (2*S*, 5*S*)-2-(4-benzyloxycarbonylamino-butyl)-3,11,18-trioxo-1,4,10-triaza-cyclooctadec-14-ene-5-carboxylic acid methyl ester (4.27)



(2*S*)-6-Benzyloxycarbonylamino-2-(pent-4-enoylamino)-hexanoic acid (4.25) N_ϵ -Cbz-*L*-Lys-OH.HCl **4.22** (5 g, 17.8 mmol, 1 equiv) was treated with 4-pentenoyl chloride **2.39** (2.33 g, 19.6 mmol, 1.1 equiv) according to General Procedure IV. The crude product was recrystallised from hot acetone/ethyl acetate and cold petroleum ether to give **4.25** (4.05 g, 63%) as a white solid.

mp = 92-95°C

^1H NMR (CDCl_3 , 500 MHz) δ 1.38 (m, 2H, $\text{CH}_2\text{CH}_2\text{CH}_2$), 1.50 (m, 2H, $\text{CH}_2\text{CH}_2\text{CH}_2$), 1.75 (m, 1H, NHCHCHH), 1.86 (m, 1H, NHCHCHH), 2.34 (m, 4H, COCH_2CH_2), 3.19 (m, 2H, CH_2NH), 4.54 (m, 1H, NHCH), 4.98-5.14 (m, 5H, $\text{CH}=\text{CH}_2$, NH , OCH_2), 5.79 (m, 1H, $\text{CH}=\text{CH}_2$), 6.59 (d, $J = 7.1$ Hz, 1H, NH), 7.32 (m, 5H, ArH).

^{13}C NMR (CDCl_3 , 75 MHz) δ 22.10, 29.12, 29.35, 31.26, 35.17, 40.30, 51.97, 66.56, 115.53, 127.82, 127.96, 128.35, 136.26, 136.56, 156.80, 173.48, 174.69.

HRMS ($\text{M}+\text{H}$) Found 363.1921 (Calcd for $\text{C}_{19}\text{H}_{27}\text{N}_2\text{O}_5$ 363.1920).

(2S, 2S)-2-[6-Benzylloxycarbonylamino-2-(pent-4-enoylamino)-hexanoylamino]-6-(pent-4-enoylamino)-hexanoic acid methyl ester (4.26) TFA salt **4.14** (250 mg, 0.70 mmol, 1 equiv) and N_α -pentenoyl- N_ϵ -Cbz-*L*-Lys-OH **4.25** (254 mg, 0.70 mmol, 1 equiv) were treated with HATU (320mg, 0.84 mmol, 1.2 equiv) according to General Procedure V. The crude product was purified by chromatography on silica (methanol/DCM, 1:9) to give **4.26** (316 mg, 77%) as an off white solid.

mp = 112–114°C

^1H NMR (CDCl_3 , 500 MHz) δ 1.28–1.55 (m, 8H, $\text{CH}_2\text{CH}_2\text{CH}_2$), 1.69 (m, 2H, NHCHCHH), 1.84 (m, 2H, NHCHCHH), 2.23–2.39 (m, 8H, COCH_2CH_2), 3.06–3.34 (m, 4H, CH_2NH), 3.66 (s, 3H, OCH_3), 4.43 (m, 2H, NHCH), 4.97–5.15 (m, 5H, $\text{CH}=\text{CH}_2$, NH), 5.06 (s, 2H, OCH_2), 5.78 (m, 2H, $\text{CH}=\text{CH}_2$), 6.00 (br s, 1H, NH), 6.46 (d, $J = 7.2$ Hz, 1H, NH), 6.92 (d, $J = 7.6$ Hz, 1H, NH), 7.32 (m, 5H, ArH).

^{13}C NMR (CDCl_3 , 75 MHz) δ 21.92, 22.25, 28.67, 28.94, 29.33, 29.47, 30.58, 31.66, 35.15, 35.40, 38.27, 40.08, 52.02, 52.06, 52.60, 66.24, 115.13, 115.31, 127.61, 127.76, 128.22, 136.41, 136.60, 136.91, 156.50, 172.07, 172.38, 172.55, 172.80.

HRMS ($\text{M}+\text{H}$) Found 587.3443 (Calcd for $\text{C}_{31}\text{H}_{47}\text{N}_4\text{O}_7$ 587.3445).

(2S, 5S)-2-(4-Benzylloxycarbonylamino-butyl)-3,11,18-trioxo-1,4,10-triaza-cyclooctadec-14-ene-5-carboxylic acid methyl ester (4.27) Diene **4.26** (146 mg, 0.25 mmol, 1 equiv) was treated with Grubbs second generation catalyst **1.17** (43 mg, 0.05 mmol, 0.2 equiv) according to General Procedure I. The crude product was purified by chromatography on silica (methanol/DCM, 1:9) and then recrystallised petroleum ether/methanol to give a mixture (38 mg, 27%) of **4.27** and double bond migrated product **4.27a** as a white solid (8:1 by ^1H NMR).

mp = 123 – 128°C (mixture)

^1H NMR (d_6 DMSO, 500 MHz) from mixture **4.27** δ 1.43 (m, 8H, CH_2), 1.58 (m, 1H, NHCHCHH), 1.68 (m, 2H, NHCHCHH), 1.79 (m, 1H, NHCHCHH), 2.20 (m, 8H, COCH_2CH_2), 3.06 (m, 4H, NHCH_2), 3.70 (s, 3H, OCH_3), 4.32 (m, 2H, NHCH), 5.09 (s, 2H, OCH_2), 5.46 (m, 2H, $\text{HC}=\text{CH}$).

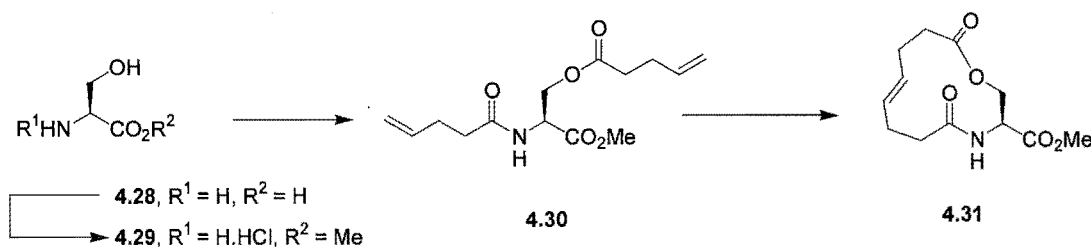
Selected data ^1H NMR (d_6 DMSO, 500 MHz) from mixture **4.27a** δ 2.87 (d, $J = 5.9$ Hz, $\text{COCH}_2\text{CH=}$), 3.13 (m, CH_2NH), 4.24 (m, NHCH), 5.14 (s, OCH_2), 5.38 (m, CH=CH).

^{13}C NMR (d_6 DMSO, 75 MHz) from mixture **4.27** δ 22.28, 22.52, 28.16, 28.19, 29.11, 30.18, 30.23, 31.36, 34.88, 35.22, 39.55, 40.15, 51.70, 51.76, 52.21, 65.05, 127.66, 128.28, 129.19, 129.24, 129.26, 137.19, 156.00, 163.26, 171.38, 172.00, 172.48.

HRMS ($\text{M}+\text{H}$) Found 559.3133 (Calcd for $\text{C}_{29}\text{H}_{43}\text{N}_4\text{O}_7$ 559.3132).

5.4.3 Serine-based cyclic compounds

Preparation of (3S)-5,12-dioxo-1-oxa-4-aza-cyclododec-8-ene-3-carboxylic acid methyl ester (**4.31**)



2-Amino-3-hydroxy-propanoic acid methyl ester hydrochloride salt (4.29) *L*-Serine **4.28** (5 g, 0.05 mol, 1 equiv) was treated with concentrated hydrochloric acid according to General Procedure II. The crude product was dissolved in a minimal amount of methanol and recrystallised with cold diethyl ether to give **4.29** (5.88 g, 80%) as a white solid.
 mp = 148-150°C

^1H NMR (D_2O , 500 MHz) δ 3.77 (s, 3H, OCH_3), 3.93 (d, $J = 3.3$ Hz, 1H, CHHOH), 4.01 (d, $J = 4.3$ Hz, 1H, CHHOH), 4.20 (t, $J = 3.8$ Hz, 1H, CH).

^{13}C NMR (D_2O , 75 MHz) δ 53.90, 54.78, 59.30, 168.90.

(2S)-Pent-4-enoic acid-[2-methoxycarbonyl-2-(pent-4-enoylamino)]-ethyl ester (**4.30**)

To a stirred solution of *L*-Ser-OMe.HCl **4.29** (200 mg, 1.7 mmol, 1 equiv) and 4-pentenoic acid (370 mg, 3.74 mmol, 2.2 equiv) in DMF (50 mL) was added DIPEA (1.05g, 8.16 mmol, 4.8 equiv) and HATU (1.53 g, 4.08 mmol, 2.4 equiv) and the solution was stirred overnight. The reaction was quenched with water (100 mL) and the resulting solution extracted with ethyl acetate (150 mL). The organic layer was then washed with aqueous

saturated sodium bicarbonate (100 mL), saturated ammonium chloride (100 mL), and saturated brine (100 mL). The resulting organic layer was dried (MgSO_4), and the solvent was removed by evaporation under reduced pressure. The crude product was purified by flash chromatography on silica (ethyl acetate/petroleum ether, 2:3) to give **4.30** (280 mg, 59%) as a pale yellow oil.

^1H NMR (CDCl_3 , 500 MHz) δ 2.35 (m, 8H, CH_2CH_2), 3.73 (s, 3H, OCH_3), 4.33 (dd, $J = 3.7, 11.5$ Hz, 1H, OCHH), 4.43 (dd, $J = 3.7, 11.5$ Hz, 1H, OCHH), 4.83 (m, 1H, NHCH), 5.01 (m, 4H, $\text{CH}=\text{CH}_2$), 5.78 (m, 2H, $\text{CH}=\text{CH}_2$), 6.32 (d, $J = 7.6$ Hz, 1H, NH).

^{13}C NMR (CDCl_3 , 75 MHz) δ 28.30, 29.01, 32.81, 34.98, 51.29, 52.44, 63.43, 115.24, 115.29, 136.10, 136.48, 169.74, 172.08, 172.22.

HRMS (M+H) Found 284.1494 (Calcd for $\text{C}_{14}\text{H}_{22}\text{NO}_5$ 284.1498).

(3S)-5,12-Dioxo-1-oxa-4-aza-cyclododec-8-ene-3-carboxylic acid methyl ester (4.31)

Diene **4.30** (110 mg, 0.39 mmol, 1 equiv) was treated with Grubbs second generation catalyst **1.17** (66 mg, 0.08 mmol, 0.2 equiv) according to General Procedure I. The crude product was purified by flash chromatography on silica (DCM/ethyl acetate, 1:1) to give **4.31** (45 mg, 45%) as brown crystals. Recrystallisation by slow evaporation from DCM/ethyl acetate gave colourless crystals suitable for x-ray crystallography. See appendix for crystallography data.

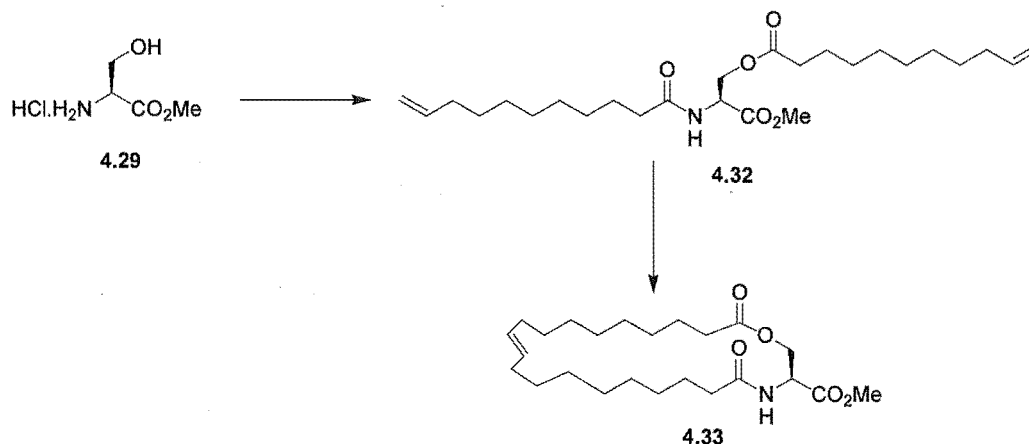
mp = 151-153°C

^1H NMR (CDCl_3 , 500 MHz) δ 2.09-2.44 (m, 8H, CH_2CH_2), 3.76 (s, 3H, OCH_3), 4.24 (dd, $J = 3.8, 11.5$ Hz, 1H, OCHH), 4.63 (m, 1H, OCHH), 4.98 (m, 1H, NHCH), 5.39 (ddt, $J = 6.3, 11.5, 15.1$ Hz, 2H, $\text{CH}=\text{CH}$), 5.92 (d, $J = 8.6$ Hz, 1H, NH).

^{13}C NMR (CDCl_3 , 75 MHz) δ 28.80, 28.85, 34.29, 36.42, 51.04, 52.51, 61.14, 130.03, 130.27, 169.61, 171.94, 172.92.

HRMS (M+H) Found 256.1183 (Calcd for $\text{C}_{12}\text{H}_{18}\text{NO}_5$ 256.1185).

Preparation of (2*S*)-5,24-dioxo-1-oxa-4-aza-cyclotetracos-14-ene-3-carboxylic acid methyl ester (4.33)



(2*S*)-Undec-10-enoic acid-[2-methoxycarbonyl-2-(undec-10-enoylamino)]-ethyl ester (4.32) To a stirred solution of *L*-Ser-OMe.HCl **4.29** (200 mg, 1.7 mmol, 1 equiv) and 10-decenoic acid (680 mg, 3.74 mmol, 2.2 equiv) in DMF (50 mL) was added DIPEA (1.05 g, 8.16 mmol, 4.8 equiv) and HATU (1.53 g, 4.08 mmol, 2.4 equiv), and the solution stirred overnight. The reaction was quenched with water (100 mL) and the resulting solution extracted with ethyl acetate (150 mL). The organic layer was washed with aqueous saturated sodium bicarbonate (100 mL), saturated ammonium chloride (100 mL), and saturated brine (100 mL), then dried (MgSO₄), and the solvent was removed by evaporation under reduced pressure. The crude product was purified by flash chromatography on silica (ethyl acetate/petroleum ether, 2:3) to give **4.32** (409 mg, 54%) as a waxy oil.

¹H NMR (CDCl₃, 500 MHz) δ 1.31 (m, 20H, CH₂), 1.58 (m, 4H, CH₂), 2.01 (m, 4H, CH₂CH=CH₂), 2.26 (m, 4H, COCH₂), 3.74 (s, 3H, OCH₃), 4.32 (dd, J = 3.5, 11.4 Hz, 1H, OCHH), 4.45 (dd, J = 4.0, 11.4 Hz, 1H, OCHH), 4.85 (m, 1H, NHCH), 4.90 (m, 2H, HC=CHH), 4.96 (dd, J = 1.3, 17.3 Hz, 2H, HC=CHH), 5.78 (m, 2H, CH=CH₂), 6.26 (d, J = 7.8 Hz, 1H, NH).

¹³C NMR (CDCl₃, 75 MHz) δ 24.47, 25.21, 28.54, 28.56, 28.72, 28.74, 28.84, 28.88, 28.97, 29.00, 33.44, 33.45, 35.58, 35.92, 51.37, 52.37, 63.26, 113.83, 113.86, 138.62, 138.65, 169.86, 172.99, 173.11.

HRMS (M+H) Found 452.3378 (Calcd for C₂₆H₄₆NO₅ 452.3376).

(2S)-5,24-Dioxo-1-oxa-4-aza-cyclotetracos-14-ene-3-carboxylic acid methyl ester (4.33)

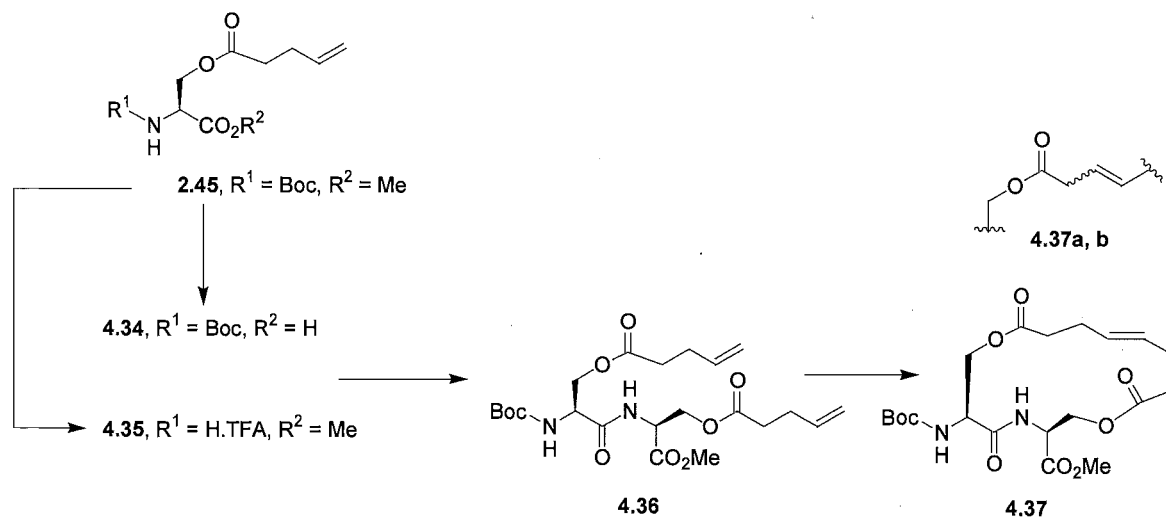
Diene **4.32** (119 mg, 0.26 mmol, 1 equiv) was treated with Grubbs second generation catalyst **1.17** (45 mg, 0.05 mmol, 0.2 equiv) according to General Procedure I. The crude product was purified by chromatography on silica (ethyl acetate/petroleum ether, 3:7) to give **4.33** (17 mg, 15%) as a brown solid.

mp = 88-90°C

^1H NMR (CDCl_3 , 500 MHz) δ 1.28 (m, 20H, CH_2), 1.59 (m, 4H, CH_2), 1.99 (m, 4H, $\text{CH}_2\text{CH}=\text{CH}_2$), 2.24 (m, 4H, COCH_2), 3.76 (s, 3H, OCH_3), 4.41 (m, 2H, OCH_2), 4.83 (m, 1H, NHCH), 5.30 (m, 2H, $\text{CH}=\text{CH}$), 6.17 (d, $J = 7.7$ Hz, 1H, NH).

^{13}C NMR (CDCl_3 , 75 MHz) δ 25.17, 25.89, 28.09, 28.76, 28.81, 28.93, 28.96, 29.04, 29.10, 29.26, 32.05, 33.89, 34.19, 36.63, 51.57, 52.81, 63.12, 130.78, 130.81, 170.02, 172.86, 173.29.

HRMS ($\text{M}+\text{H}$) Found 424.3068 (Calcd for $\text{C}_{24}\text{H}_{42}\text{NO}_5$ 424.3063).

Preparation of (3S, 6S)-6-tert-butoxycarbonylamino-5,9,16-trioxo-1,8-dioxa-4-azacyclohexadec-12-ene-3-carboxylic acid methyl ester (4.37)

(1S, 2S)-Pent-4-enoic acid {2-tert-butoxycarbonylamino-2-[(1-methoxycarbonyl-2-(pent-4-enoyloxy)-ethylcarbamoyl]}-ethyl ester (4.36) Boc-*L*-Ser-OMe **2.45** (300 mg, 1 mmol) was treated with TFA according to General Procedure VI to give TFA salt **4.35**. In a separate reaction, Boc-*L*-Ser-OMe **2.45** (300 mg, 1 mmol) was treated with 0.2 M lithium hydroxide (2 equiv) according to General Procedure VIII to give unprotected carboxylic acid **4.34**. Compounds **4.35** and **4.34** (assume each 1 mmol, 1 equiv) were then treated

with EDCI (248mg, 1.3 mmol, 1.3 equiv) and HOBt (227mg, 1.5 mmol, 1.5 equiv) according to General Procedure VII. The crude product was purified by flash chromatography on silica (ethyl acetate/DCM, 3:7) to give **4.36** (60 mg, 13%) as a pale yellow oil.

¹H NMR (CDCl₃, 500 MHz) δ 1.42 (s, 9H, C(CH₃)₃), 2.37 (m, 8H, COCH₂CH₂), 3.74 (s, 3H, OCH₃), 4.24-4.44 (m, 5H, OCH₂, NHCH), 4.78 (m, 1H, NHCH), 5.00 (m, 4H, CH=CH₂), 5.32 (br d, *J* = 5.7 Hz, 1H, NH), 5.77 (m, 2H, CH=CH₂), 7.04 (d, *J* = 7.6 Hz, 1H, NH).

¹³C NMR (CDCl₃, 75 MHz) δ 28.01, 28.37, 28.42, 32.87, 32.97, 51.68, 52.65, 53.21, 63.35, 63.65, 80.24, 115.42, 115.45, 136.15, 136.30, 155.13, 169.01, 169.18, 172.26, 172.49.

HRMS (M+H) Found 471.2347 (Calcd for C₂₂H₃₅N₂O₉ 471.2343).

(3*S*, 6*S*)-6-*tert*-Butoxycarbonylamino-5,9,16-trioxo-1,8-dioxo-4-aza-cyclohexadec-12-ene-3-carboxylic acid methyl ester (4.37) Diene **4.36** (40 mg, 0.09 mmol, 1 equiv) was treated with Grubbs second generation catalyst **1.17** (15 mg, 0.02 mmol, 0.2 equiv) according to General Procedure I. The crude product was purified by chromatography on silica (ethyl acetate/DCM, 3:7) to give a mixture (17 mg, 45%) of **4.37** and the double bond migrated products **4.37a** and **b** as an off white solid (23:1:1 by ¹H NMR).

mp = 141-143°C (mixture)

¹H NMR (CDCl₃, 500 MHz) from mixture **4.37** δ 1.46 (s, 9H, C(CH₃)₃), 2.29-2.54 (m, 4H, COCH₂CH₂), 3.76 (s, 3H, OCH₃), 4.31 (m, 1H, OCHH), 4.30-4.39 (m, 3H, OCH₂, NHCH), 4.58 (dd, *J* = 3.1, 11.4 Hz, 1H, OCHH), 4.80 (br s, 1H, NHCH), 5.46 (m, 2H, HC=CH), 5.58 (br s, 1H, NH), 7.17 (d, *J* = 7.2 Hz, 1H, NH).

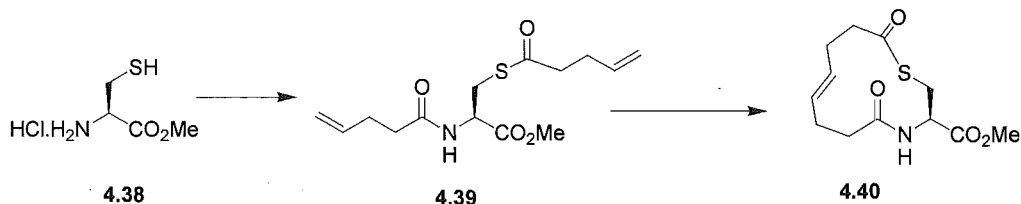
Selected data ¹H NMR (CDCl₃, 500 MHz) from mixture **4.37a** and **b** δ 2.97 (d, *J* = 6.2 Hz, COCH₂CH=), 3.03 (d, *J* = 4.4 Hz, COCH₂CH=), 4.51 (m, OCHH), 5.32 (m, CH=CH).

¹³C NMR (CDCl₃, 75 MHz) from mixture **4.37** 27.01, 27.14, 28.14, 33.37, 33.48, 52.06, 52.82, 54.26, 64.14, 64.26, 80.73, 128.74, 129.30, 155.14, 169.01, 169.06, 172.60, 173.55.

HRMS (M+H) Found 443.2028 (Calcd for C₂₀H₃₁N₂O₉ 443.2030).

5.4.4 Cysteine-based cyclic compounds

Preparation of (3*R*)-5,12-dioxo-1-thia-4-aza-cyclododec-8-ene-3-carboxylic acid methyl ester (4.40)



(2*R*)-2-(Pent-4-enoylamino)-3-(pent-4-enoylsulfanyl)-propionic acid methyl ester (4.39)

To a stirred solution of 4-pentenoic acid (321 mg, 3.2 mmol, 2.2 equiv) in dry DCM (10 mL) under an inert atmosphere was added dropwise TEA (876 mg, 8.6 mmol, 5.9 equiv) at 0°C. After stirring for 20 min, this solution was added dropwise to a solution of BOP-Cl (890 mg, 3.5 mmol, 2.4 equiv) in dry DCM (20 mL) at 0°C. A solution of *L*-Cys-OMe.HCl **4.38** (250 mg, 1.5 mmol, 1 equiv) in dry DCM (10 mL) was then added dropwise at 0°C. After 15 min the reaction was allowed to warm to rt with stirring overnight. The solvent was removed by evaporation under reduced pressure and the residue dissolved in ethyl acetate (50 mL). The solution was washed twice with water (30 mL), and then the organic layer washed twice with saturated aqueous sodium bicarbonate (30 mL), water (30 mL), and saturated brine solution (30 mL) sequentially. The resulting organic layer was dried (MgSO₄), and the solvent was removed by evaporation under reduced pressure. The crude product was purified by flash chromatography on silica (ethyl acetate/petroleum ether, 1:4) to give **4.39** (372 mg, 85%) as a colourless oil.

¹H NMR (CDCl₃, 500 MHz) δ 2.27 (m, 2H, COCH₂), 2.36 (m, 4H, =CHCH₂), 2.63 (t, *J* = 7.3 Hz, 2H, COCH₂), 3.35 (m, 2H, CH₂S), 3.72 (s, 3H, OCH₃), 4.77 (m, 1H, NHCH), 4.97-5.06 (m, 4H, CH=CH₂), 5.77 (m, 2H, CH=CH₂), 6.28 (br d, *J* = 7.1 Hz, 1H, NH).

¹³C NMR (CDCl₃, 75 MHz) δ 28.53, 28.70, 29.67, 34.42, 42.19, 51.26, 51.84, 114.62, 115.22, 135.27, 136.31, 167.00, 171.84, 196.97.

HRMS (M+H) Found 300.1273 (Calcd for C₁₄H₂₂NO₄S 300.1270).

(3*R*)-5,12-Dioxo-1-thia-4-aza-cyclododec-8-ene-3-carboxylic acid methyl ester (4.40)

Diene **4.39** (34 mg, 0.11 mmol, 1 equiv) was treated with Grubbs second generation catalyst **1.17** (19 mg, 0.02 mmol, 0.2 equiv) according to General Procedure I. The crude

product was purified by flash chromatography on silica (petroleum ether/ethyl acetate, 1:4) to give **4.40** (23 mg, 74%) as a white solid. Recrystallisation by slow evaporation from DCM/ethyl acetate gave crystals suitable for x-ray crystallography. See appendix for crystallography data.

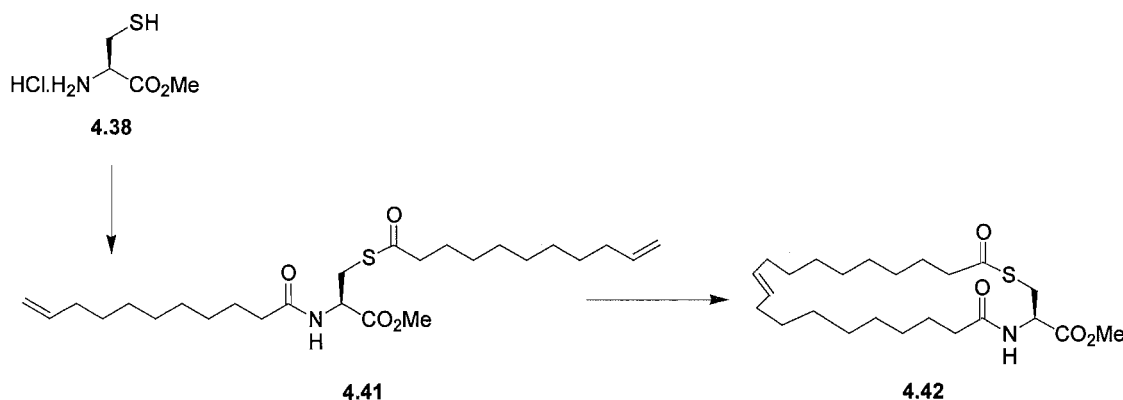
mp = 146-147°C

^1H NMR (CDCl_3 , 500 MHz) δ 2.02 (m, 1H, COCHHCH_2), 2.26-2.43 (m, 5H, $=\text{CHCH}_2$, COCHHCH_2), 2.62 (m, 1H, COCHHCH_2), 2.69 (m, 1H, COCHHCH_2), 3.45 (m, 2H, CH_2S), 3.74 (s, 3H, OCH_3), 4.80 (m, 1H, NHCH), 5.33 (m, 1H, $\text{CH}=\text{CH}$), 5.41 (m, 1H, $\text{CH}=\text{CH}$), 5.85 (br s, 1H, NH).

^{13}C NMR (CDCl_3 , 75 MHz) δ 29.01, 29.36, 29.50, 37.48, 42.74, 51.92, 52.64, 128.20, 131.81, 170.77, 172.40, 199.12.

HRMS ($\text{M}+\text{H}$) Found 272.0960 (Calcd for $\text{C}_{12}\text{H}_{18}\text{NO}_4$ 272.0957).

Preparation of (3*R*)-5,24-dioxo-1-thia-4-aza-cyclotetracos-14-ene-3-carboxylic acid methyl ester (**4.42**)



(2*R*)-2-(Undec-10-enoylamino)-3-(undec-10-enoylsulfanyl)-propanoic acid methyl ester (4.41**)** To a stirred solution of 10-decenoic acid (590 mg, 3.2 mmol, 2.2 equiv) in dry DCM (10 mL) under an inert atmosphere was added dropwise TEA (876 mg, 8.6 mmol, 5.9 equiv) at 0°C. After stirring for 20 min, this solution was added dropwise to a solution of BOP-Cl (890 mg, 3.5 mmol, 2.4 equiv) in dry DCM (20 mL) at 0°C. A solution of *L*-Cys-OMe.HCl **4.38** (250 mg, 1.5 mmol, 1 equiv) in dry DCM (10 mL) was then added at 0°C. After 15 min the reaction was allowed to warm to rt with stirring overnight. The solvent was removed by evaporation under reduced pressure and the residue dissolved in ethyl acetate (50 mL). The solution was washed twice with water (30 mL), and then the

organic layer washed twice with saturated aqueous sodium bicarbonate (30 mL), water (30 mL), and saturated brine solution (30 mL) sequentially. The resulting organic layer was dried (MgSO_4), and the solvent was removed by evaporation under reduced pressure. The crude product was purified by flash chromatography on silica (ethyl acetate/petroleum ether, 1:1) to give **4.41** (300 mg, 44%) as a white solid.

mp = 40-41°C

^1H NMR (CDCl_3 , 500 MHz) δ 1.30 (m, 20H, CH_2), 1.59 (m, 4H, CH_2), 2.00 (m, 4H, $\text{CH}_2\text{CH}=\text{CH}_2$), 2.16 (t, $J = 7.6$ Hz, 2H, NHCOCH_2), 2.52 (t, $J = 7.5$ Hz, 2H, SCOCH_2), 3.32 (d, $J = 5.5$ Hz, 2H, CH_2S), 3.71 (s, 3H, OCH_3), 4.76 (m, 1H, NHCH), 4.89 (m, 2H, $\text{CH}=\text{CH}_2$), 4.95 (ddd, $J = 1.6, 3.4, 17.1$ Hz, 2H, $\text{CH}=\text{CH}_2$), 5.77 (m, 2H, $\text{CH}=\text{CH}_2$), 6.24 (d, $J = 7.5$ Hz, 1H, NH).

^{13}C NMR (CDCl_3 , 75 MHz) δ 25.18, 25.25, 28.54, 28.57, 28.70, 28.76, 28.85, 28.92, 29.00, 29.01, 30.16, 33.44, 33.46, 35.97, 43.60, 51.70, 52.28, 113.85, 113.89, 138.62, 138.67, 170.47, 172.76, 198.53.

HRMS (M+H) Found 468.3166 (Calcd for $\text{C}_{26}\text{H}_{46}\text{NO}_4\text{S}$ 468.3148).

(3R)-5,24-Dioxo-1-thia-4-aza-cyclotetracos-14-ene-3-carboxylic acid methyl ester (4.42)

Diene **4.41** (50 mg, 0.11 mmol, 1 equiv) was treated with Grubbs second generation catalyst **1.17** (18 mg, 0.02 mmol, 0.2 equiv) according to General Procedure I. The crude product was purified by chromatography on silica (ethyl acetate/petroleum ether, 3:7) to give **4.42** (20 mg, 43%) as a white solid.

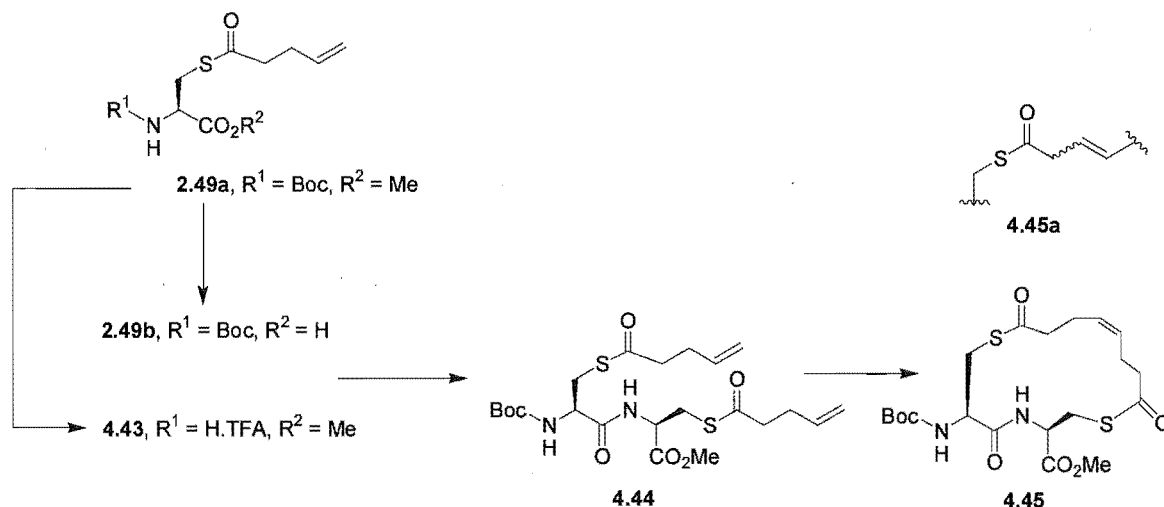
mp = 94-96°C

^1H NMR (CDCl_3 , 500 MHz) δ 1.30 (m, 16H, CH_2), 1.60 (m, 8H, CH_2), 2.00 (m, 4H, $\text{CH}_2\text{CH}=\text{CH}_2$), 2.16 (m, 2H, NHCOCH_2), 2.55 (m, 2H, SCOCH_2), 3.33 (m, 2H, SCH_2), 3.75 (s, 3H, OCH_3), 4.77 (m, 1H, NHCH), 5.31 (m, 2H, $\text{CH}=\text{CH}$), 6.16 (d, $J = 8.0$ Hz, 1H, NH).

^{13}C NMR (CDCl_3 , 75 MHz) δ 25.91, 25.20, 27.94, 28.23, 28.65, 28.67, 28.81, 28.92, 29.20, 29.37, 29.44, 29.57, 30.01, 31.99, 32.13, 36.80, 43.90, 52.25, 52.73, 130.76, 130.94, 170.77, 173.13, 199.92.

HRMS (M+H) Found 440.2843 (Calcd for $\text{C}_{24}\text{H}_{42}\text{NO}_4\text{S}$ 440.2835).

Preparation of (3*R*, 6*R*)-6-*tert*-butoxycarbonylamino-5,9,16-trioxo-1,8-dithia-4-azacyclohexadec-12-ene-3-carboxylic acid methyl ester (4.45)



(2*R*, 2*R*)-2-[2-*tert*-Butoxycarbonylamino-3-(pent-4-enoylsulfanyl)-propionylamino]-3-(pent-4-enoylsulfanyl)-propionic acid methyl ester (4.44) Boc-*L*-Cys-OMe **2.49a** (700 mg, 2.2 mmol) was treated with TFA according to General Procedure VI to give TFA salt **4.43**. In a separate reaction, Boc-*L*-Cys-OMe **2.49a** (700 mg, 2.2 mmol) was treated with 0.2 M lithium hydroxide (2 equiv) according to General Procedure VIII to give unprotected carboxylic acid **2.49b**. Compounds **4.43** and **2.49b** (assume each 2.2 mmol, 1 equiv) were treated with HATU (456 mg, 1.2 mmol, 1.2 equiv) according to General Procedure V. The crude product was purified twice by flash chromatography on silica (ethyl acetate/DCM, 1:4 then 3:7) to give **4.44** (90 mg, 11%) as a pale yellow oil.

¹H NMR (CDCl₃, 500 MHz) δ 1.44 (s, 9H, C(CH₃)₃), 2.29-2.43 (m, 6H, CH₂), 2.68 (t, *J* = 7.4 Hz, 2H, COCH₂), 3.20-3.46 (m, 4H, SCH₂), 3.72 (s, 3H, OCH₃), 4.43 (m, 1H, CHNH), 4.85 (m, 1H, CHNH), 5.03 (m, 4H, CH=CH₂), 5.31 (br d, *J* = 7.6 Hz, 1H, NH), 5.79 (m, 2H, CH=CH₂), 6.33 (br d, *J* = 7.6 Hz, 1H, NH).

¹³C NMR (CDCl₃, 75 MHz) δ 28.12, 29.11, 29.21, 30.06, 30.27, 35.14, 42.87, 51.40, 52.59, 60.60, 80.54, 115.38, 116.07, 135.53, 136.73, 155.13, 170.42, 172.14, 198.66, 199.43.

HRMS (M+H) Found 503.1895 (Calcd for C₂₂H₃₅N₂O₇S₂ 503.1886).

(3*R*, 6*R*)-6-*tert*-Butoxycarbonylamino-5,9,16-trioxo-1,8-dithia-4-aza-cyclohexadec-12-ene-3-carboxylic acid methyl ester (4.45) Diene **4.44** (53 mg, 0.11 mmol, 1 equiv) was treated with Grubbs second generation catalyst **1.17** (18 mg, 0.01 mmol, 0.2 equiv) according to General Procedure I. The crude product was purified by chromatography on silica (ethyl acetate/DCM, 2:3) to give a mixture (18 mg, 36%) of **4.45** and the double bond migrated product **4.45a** as a thick, brown oil (10:1 by ^1H NMR).

^1H NMR (CDCl_3 , 500 MHz) from mixture **4.45** δ 1.43 (s, 9H, $\text{C}(\text{CH}_3)_3$), 2.23 (m, 2H, $=\text{CHCH}_2$), 2.39 (m, 4H, $=\text{CHCH}_2$, COCH_2), 2.70 (m, 2H, COCH_2), 3.35 (m, 4H, SCH_2), 3.74 (s, 3H, OCH_3), 4.73 (m, 2H, CHNH), 5.55 (m, 2H, $\text{CH}=\text{CH}$), 5.66 (br d, $J = 7.9$ Hz, 1H, NH), 6.42 (br d, $J = 7.0$ Hz, 1H, NH).

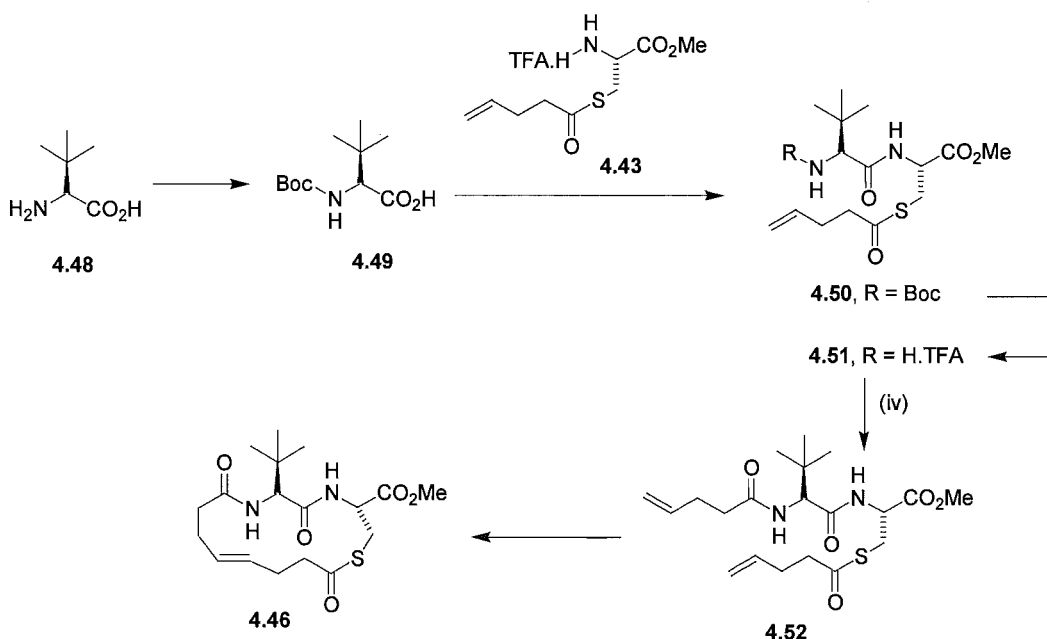
Selected data ^1H NMR (CDCl_3 , 500 MHz) from mixture **4.45a** δ 2.99 (d, $J = 7.3$ Hz, $\text{COCH}_2\text{CH}=\text{}$), 4.80 (m, CHNH), 5.34 (m, $\text{CH}=\text{CH}$), 6.35 (br d, $J = 8.1$ Hz, NH).

^{13}C NMR (CDCl_3 , 75 MHz) from mixture **4.45** δ 27.50, 28.18, 28.22, 30.47, 31.08, 35.33, 42.70, 52.36, 52.73, 59.17, 80.75, 129.24, 130.90, 154.86, 170.38, 172.65, 199.11, 200.10.

HRMS ($\text{M}+\text{H}$) Found 475.1577 (Calcd for $\text{C}_{20}\text{H}_{31}\text{N}_2\text{O}_7\text{S}_2$ 475.1573).

5.4.5 Synthesis of cysteine-*tert*-leucine-based dipeptide

Preparation of (3*S*, 6*R*) 6-*tert*-butyl-5,8,15-trioxo-1-thia-4,7-diaza-cyclopentadec-11-ene-3-carboxylic acid methyl ester (4.46)



(2S)-2-tert-Butoxycarbonylamino-3,3-dimethyl-butyric acid (4.49)¹⁸ Boc₂O (182 mg, 0.84 mmol, 1.1 equiv) was added to a solution of *L*-tert-leucine **4.48** (100 mg, 0.76 mmol, 1 equiv) and NaOH (34 mg, 0.84 mmol, 1.1 equiv) in water/*tert*-butyl alcohol (1 mL/1 mL). The reaction mixture was stirred at rt overnight, and was extracted three times with ethyl acetate (5 mL). The combined organic layers were washed with saturated aqueous sodium bicarbonate solution, and the combined water phases were acidified to pH 1.5-2.0 (Universal indicator paper). The water phase was extracted four times with ethyl acetate (5 mL), and the combined organic phases were washed with saturated brine solution, dried (MgSO₄), and the solvent removed by evaporation under reduced pressure to give **4.49** (175 mg, 99%) as a colourless solid.

mp = 122-123°C

¹H NMR (CDCl₃, 500 MHz) δ 1.01 (s, 9H, C(CH₃)₃_{leu}), 1.43 (s, 9H, C(CH₃)₃_{boc}), 4.11 (d, *J* = 7.2 Hz, 1H, CH), 5.08 (d, *J* = 9.3 Hz, 1H, NH).

¹³C NMR (CDCl₃, 75 MHz) δ 26.41, 28.20, 34.41, 61.55, 79.83, 155.62, 176.22.

(2S, 2R) 2-(2-tert-Butoxycarbonylamino-3,3-dimethyl-butyrylamino)-3-(pent-4-enoyl-sulfanyl)-propionic acid methyl ester (4.50) *N*_ε-Pentenoyl cysteine **2.49a** (1 g, 3.15 mmol, 1 equiv) was treated with TFA according to General Procedure VI to give **4.43**. TFA salt **4.43** (3.15 mmol) and Boc-*L*-leu-OH **4.49** (831 mg, 3.6 mmol, 1.14 equiv) were treated with EDCI (782 mg, 4.1 mmol, 1.3 equiv) and HOBt (713 mg, 4.73 mmol, 1.5 equiv) according to General Procedure VII. The crude product was purified by flash chromatography on silica (ethyl acetate/DCM, 1:4) to give **4.50** (620 mg, 46%) as a pale yellow oil.

¹H NMR (CDCl₃, 500 MHz) δ 0.99 (s, 9H, C(CH₃)₃_{leu}), 1.43 (s, 9H, C(CH₃)₃_{boc}), 2.29 (m, 2H, COCH₂CH₂), 2.36 (t, *J* = 7.0 Hz, 2H, COCH₂CH₂), 3.30 (dd, *J* = 5.0, 14.2 Hz, 1H, SCHH), 3.46 (dd, *J* = 4.6, 14.2 Hz, 1H, SCHH), 3.70 (s, 3H, OCH₃), 4.02 (d, *J* = 8.3 Hz, 1H, CH_{leu}), 4.86 (m, 1H, CH_{cys}), 4.98 (d, *J* = 10.1 Hz, 1H, NH_{leu}), 5.04 (m, 2H, CH=CH₂), 5.81 (m, 1H, CH=CH₂), 6.38 (d, *J* = 7.7 Hz, 1H, NH_{cys}).

¹³C NMR (CDCl₃, 75 MHz) δ 26.71, 28.19, 29.15, 30.09, 33.79, 35.16, 51.53, 52.52, 68.62, 80.37, 115.33, 136.76, 155.49, 170.59, 172.32, 199.50.

HRMS Found (M+H) 431.2214 (Calcd for C₂₀H₃₅N₂O₆S 431.2216).

(2S, 2R) 2-[3,3-Dimethyl-2-(pent-4-enoylamino)-butyrylamino]-3-(pent-4-enoylsulfanyl)-propionic acid methyl ester (4.52) Dipeptide **4.50** (620 mg, 1.44 mmol, 1 equiv) was treated with TFA according to General Procedure VI to give **4.51**. TFA salt **4.51** (1.44 mmol) and 4-pentenoic acid (164 mg, 1.6 mmol, 1.14 equiv) were treated with EDCI (358 mg, 1.87 mmol, 1.3 equiv) and HOBt (326 mg, 2.16 mmol, 1.5 equiv) according to General Procedure VII. The crude product was purified by flash chromatography on silica (ethyl acetate/DCM, 1:4) to give **4.52** (430 mg, 72%) as a colourless oil.

^1H NMR (CDCl_3 , 500 MHz) δ 0.99 (s, 9H, $\text{C}(\text{CH}_3)_3$), 2.35 (m, 4H, COCH_2CH_2), 3.28 (dd, $J = 5.4, 14.2$ Hz, 1H, SCHH), 3.46 (dd, $J = 4.6, 14.2$ Hz, 1H, SCHH), 3.72 (s, 3H, OCH_3), 4.42 (d, $J = 8.6$ Hz, 1H, CH_{Ieu}), 4.83 (m, 1H, CH_{cys}), 4.98-5.11 (m, 4H, $\text{CH}=\text{CH}_2$), 5.82 (m, 2H, $\text{CH}=\text{CH}_2$), 6.05 (d, $J = 8.6$ Hz, 1H, NH_{Ieu}), 6.35 (d, $J = 7.7$ Hz, 1H, NH_{cys}).

^{13}C NMR (CDCl_3 , 75 MHz) δ 26.44, 28.39, 28.92, 29.82, 32.88, 33.54, 34.73, 51.50, 52.18, 67.01, 115.00, 115.12, 136.43, 136.62, 170.23, 172.30, 173.06, 198.57.

HRMS Found (M+H) 413.2115 (Calcd for $\text{C}_{20}\text{H}_{32}\text{N}_2\text{O}_5\text{S}$ 413.2110).

(3S, 6R) 6-tert-Butyl-5,8,15-trioxo-1-thia-4,7-diaza-cyclopentadec-11-ene-3-carboxylic acid methyl ester (4.46) Diene **4.52** (230 mg, 0.60 mmol, 1 equiv) was treated with Grubbs second generation catalyst **1.17** (102 mg, 0.12 mmol, 0.2 equiv) according to General Procedure I. The crude product was purified by flash chromatography on silica (100% ethyl acetate) to give **4.52** (140 mg, 65%) as white solid.

^1H NMR (CDCl_3 , 500 MHz) δ 0.99 (s, 9H, $\text{C}(\text{CH}_3)_3$), 2.20 (m, 4H, $=\text{CHCH}_2$ (3H), COCH_2 (1H)), 2.44 (m, 3H, $=\text{CHCH}_2$ (1H), COCH_2 (2H)), 2.57 (m, 2H, COCH_2), 3.11 (dd, $J = 3.9, 14.5$ Hz, 1H, SCHH), 3.46 (dd, $J = 9.5, 14.5$ Hz, 1H, SCHH), 3.73 (s, 3H, OCH_3), 4.59 (m, 2H, NHCH), 5.52 (m, 2H, $\text{HC}=\text{CH}$), 5.86 (d, $J = 9.7$ Hz, 1H, NH), 6.41 (d, $J = 7.4$ Hz, 1H, NH).

^{13}C NMR (CDCl_3 , 75 MHz) δ 26.48, 27.22, 27.63, 29.61, 34.10, 35.43, 35.73, 52.67, 52.90, 65.47, 129.71, 130.24, 170.84, 172.29, 172.45, 200.16.

HRMS Found (M + H) 385.1796 (Calcd for $\text{C}_{18}\text{H}_{29}\text{N}_2\text{O}_5\text{S}$ 385.1797).

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APPENDIX

Crystallographic data for twelve membered-cysteine ring 4.40

Empirical formula	C ₁₂ H ₁₇ N O ₄ S
Formula weight	271.33
Temperature	93(2) K
Wavelength	0.71073 Å
Crystal system, space group	Orthorhombic, P2(1)2(1)2(1)
Unit cell dimensions	a = 4.9843(6) Å alpha = 90 deg. b = 15.5996(18) Å beta = 90 deg. c = 16.708(2) Å gamma = 90 deg.
Volume	1299.1(3) Å ³
Z, Calculated density	4, 1.387 Mg/m ³
Absorption coefficient	0.256 mm ⁻¹
F(000)	576
Crystal size	0.62 x 0.43 x 0.13 mm
Theta range for data collection	2.44 to 26.36 deg.
Limiting indices	-5<=h<=6, -19<=k<=19, -17<=l<=20
Reflections collected / unique	6179 / 2641 [R(int) = 0.0368]
Completeness to theta = 26.36	99.7 %
Max. and min. transmission	0.9675 and 0.8576
Refinement method	Full-matrix least-squares on F ²
Data / restraints / parameters	2641 / 0 / 168
Goodness-of-fit on F ²	1.029
Final R indices [I>2sigma(I)]	R1 = 0.0324, wR2 = 0.0694
R indices (all data)	R1 = 0.0421, wR2 = 0.0724
Absolute structure parameter	-0.02(8)
Largest diff. peak and hole	0.192 and -0.235 e.Å ⁻³

Table 1. Crystal data and structure refinement for 4.40.

	x	y	z	U(eq)
S	1956(1)	10021(1)	7826(1)	16(1)
N	4259(3)	8342(1)	6971(1)	13(1)
C(1)	3812(4)	9711(1)	8681(1)	15(1)
C(2)	2170(4)	9708(1)	9441(1)	15(1)
C(3)	2156(5)	8812(1)	9816(1)	19(1)
C(4)	983(4)	8168(1)	9264(1)	18(1)
C(5)	2344(4)	7535(1)	8919(1)	16(1)
C(6)	1237(4)	6922(1)	8318(1)	17(1)
C(7)	2674(4)	6993(1)	7512(1)	14(1)
C(8)	2123(4)	7839(1)	7105(1)	12(1)
C(9)	4070(4)	9143(1)	6533(1)	13(1)
C(10)	4484(4)	9928(1)	7062(1)	15(1)
C(11)	6138(4)	9153(1)	5865(1)	13(1)
C(12)	7757(5)	8406(1)	4743(1)	25(1)
O(1)	6142(3)	9517(1)	8659(1)	24(1)
O(2)	-171(3)	8039(1)	6901(1)	17(1)
O(3)	5826(3)	8479(1)	5381(1)	19(1)
O(4)	7820(3)	9699(1)	5769(1)	16(1)

Table 2. Atomic coordinates ($\times 10^4$) and equivalent isotropic displacement parameters ($\text{\AA}^2 \times 10^3$) for **4.40**. U(eq) is defined as one third of the trace of the orthogonalized Uij tensor.

S-C(1)	1.770(2)	C(6)-H(6A)	0.9900
S-C(10)	1.7989(18)	C(6)-H(6B)	0.9900
N-C(8)	1.342(2)	C(7)-C(8)	1.510(3)
N-C(9)	1.450(2)	C(7)-H(7A)	0.9900
N-H	0.83(2)	C(7)-H(7B)	0.9900
C(1)-O(1)	1.201(2)	C(8)-O(2)	1.233(2)
C(1)-C(2)	1.511(3)	C(9)-C(11)	1.520(3)
C(2)-C(3)	1.531(3)	C(9)-C(10)	1.523(3)
C(2)-H(2A)	0.9900	C(9)-H(9)	1.0000
C(2)-H(2B)	0.9900	C(10)-H(10A)	0.9900
C(3)-C(4)	1.485(3)	C(10)-H(10B)	0.9900
C(3)-H(3A)	0.9900	C(11)-O(4)	1.205(2)
C(3)-H(3B)	0.9900	C(11)-O(3)	1.335(2)
C(4)-C(5)	1.329(3)	C(12)-O(3)	1.441(3)
C(4)-H(4)	0.9500	C(12)-H(12A)	0.9800
C(5)-C(6)	1.492(3)	C(12)-H(12B)	0.9800
C(5)-H(5)	0.9500	C(12)-H(12C)	0.9800
C(6)-C(7)	1.529(3)		

Table 3. Bond lengths [Å] for **4.40**. Symmetry transformations used to generate equivalent atoms.

C(1)-S-C(10)	100.63(9)	C(8)-C(7)-C(6)	111.97(16)
C(8)-N-C(9)	122.46(17)	C(8)-C(7)-H(7A)	109.2
C(8)-N-H	121.2(14)	C(6)-C(7)-H(7A)	109.2
C(9)-N-H	116.1(14)	C(8)-C(7)-H(7B)	109.2
O(1)-C(1)-C(2)	123.33(19)	C(6)-C(7)-H(7B)	109.2
O(1)-C(1)-S	123.31(16)	H(7A)-C(7)-H(7B)	107.9
C(2)-C(1)-S	113.37(14)	O(2)-C(8)-N	122.78(17)
C(1)-C(2)-C(3)	110.45(15)	O(2)-C(8)-C(7)	120.96(17)
C(1)-C(2)-H(2A)	109.6	N-C(8)-C(7)	116.25(17)
C(3)-C(2)-H(2A)	109.6	N-C(9)-C(11)	109.60(16)
C(1)-C(2)-H(2B)	109.6	N-C(9)-C(10)	113.02(16)
C(3)-C(2)-H(2B)	109.6	C(11)-C(9)-C(10)	109.02(15)
H(2A)-C(2)-H(2B)	108.1	N-C(9)-H(9)	108.4
C(4)-C(3)-C(2)	111.39(16)	C(11)-C(9)-H(9)	108.4
C(4)-C(3)-H(3A)	109.4	C(10)-C(9)-H(9)	108.4
C(2)-C(3)-H(3A)	109.4	C(9)-C(10)-S	112.43(13)
C(4)-C(3)-H(3B)	109.4	C(9)-C(10)-H(10A)	109.1
C(2)-C(3)-H(3B)	109.4	S-C(10)-H(10A)	109.1
H(3A)-C(3)-H(3B)	108.0	C(9)-C(10)-H(10B)	109.1
C(5)-C(4)-C(3)	124.8(2)	S-C(10)-H(10B)	109.1
C(5)-C(4)-H(4)	117.6	H(10A)-C(10)-H(10B)	107.9
C(3)-C(4)-H(4)	117.6	O(4)-C(11)-O(3)	123.86(18)
C(4)-C(5)-C(6)	125.3(2)	O(4)-C(11)-C(9)	125.19(17)
C(4)-C(5)-H(5)	117.3	O(3)-C(11)-C(9)	110.94(16)
C(6)-C(5)-H(5)	117.3	O(3)-C(12)-H(12A)	109.5
C(5)-C(6)-C(7)	111.93(16)	O(3)-C(12)-H(12B)	109.5
C(5)-C(6)-H(6A)	109.2	H(12A)-C(12)-H(12B)	109.5
C(7)-C(6)-H(6A)	109.2	O(3)-C(12)-H(12C)	109.5
C(5)-C(6)-H(6B)	109.2	H(12A)-C(12)-H(12C)	109.5
C(7)-C(6)-H(6B)	109.2	H(12B)-C(12)-H(12C)	109.5
H(6A)-C(6)-H(6B)	107.9	C(11)-O(3)-C(12)	115.64(16)

Table 4. Bond angles [deg] for **4.40**.

	U11	U22	U33	U23	U13	U12
S	16(1)	19(1)	14(1)	2(1)	2(1)	4(1)
N	9(1)	14(1)	15(1)	4(1)	-1(1)	3(1)
C(1)	16(1)	12(1)	16(1)	-3(1)	-1(1)	-3(1)
C(2)	17(1)	16(1)	14(1)	-4(1)	-1(1)	0(1)
C(3)	24(1)	21(1)	13(1)	0(1)	3(1)	1(1)
C(4)	19(1)	17(1)	18(1)	4(1)	4(1)	-3(1)
C(5)	17(1)	18(1)	14(1)	7(1)	1(1)	0(1)
C(6)	20(1)	15(1)	16(1)	3(1)	3(1)	0(1)
C(7)	15(1)	12(1)	16(1)	0(1)	0(1)	-1(1)
C(8)	13(1)	11(1)	11(1)	-4(1)	1(1)	0(1)
C(9)	14(1)	12(1)	12(1)	3(1)	-1(1)	0(1)
C(10)	19(1)	14(1)	14(1)	3(1)	3(1)	-1(1)
C(11)	14(1)	12(1)	11(1)	2(1)	-2(1)	3(1)
C(12)	37(1)	18(1)	18(1)	-3(1)	10(1)	0(1)
O(1)	14(1)	39(1)	18(1)	0(1)	-1(1)	2(1)
O(2)	12(1)	18(1)	21(1)	3(1)	-1(1)	-1(1)
O(3)	28(1)	15(1)	15(1)	-3(1)	4(1)	-4(1)
O(4)	17(1)	15(1)	17(1)	1(1)	2(1)	-2(1)

Table 5. Anisotropic displacement parameters ($\text{\AA}^2 \times 10^3$) for **4.40**. The anisotropic displacement factor exponent takes the form: $-2 \pi^2 [h^2 a^{*2} U_{11} + \dots + 2 h k a^* b^* U_{12}]$.

	x	y	z	U(eq)
H	5790(40)	8187(13)	7095(12)	8(5)
H(2A)	307	9885	9320	18
H(2B)	2935	10124	9826	18
H(3A)	4017	8644	9952	23
H(3B)	1103	8825	10318	23
H(4)	-878	8211	9148	22
H(5)	4172	7468	9067	19
H(6A)	-698	7039	8241	20
H(6B)	1421	6330	8524	20
H(7A)	4631	6930	7596	17
H(7B)	2077	6519	7160	17
H(9)	2244	9180	6288	15
H(10A)	4456	10449	6724	18
H(10B)	6271	9891	7319	18
H(12A)	7625	8908	4393	37
H(12B)	7390	7886	4432	37
H(12C)	9568	8373	4969	37

Table 6. Hydrogen coordinates ($\times 10^4$) and isotropic displacement parameters ($\text{\AA}^2 \times 10^3$) for **4.40**.

C(10)-S-C(1)-O(1)	0.8(2)
C(10)-S-C(1)-C(2)	-178.85(13)
O(1)-C(1)-C(2)-C(3)	-58.9(3)
S-C(1)-C(2)-C(3)	120.71(16)
C(1)-C(2)-C(3)-C(4)	-60.1(2)
C(2)-C(3)-C(4)-C(5)	112.3(2)
C(3)-C(4)-C(5)-C(6)	-175.35(17)
C(4)-C(5)-C(6)-C(7)	117.7(2)
C(5)-C(6)-C(7)-C(8)	-67.5(2)
C(9)-N-C(8)-O(2)	-3.7(3)
C(9)-N-C(8)-C(7)	175.36(17)
C(6)-C(7)-C(8)-O(2)	-62.6(2)
C(6)-C(7)-C(8)-N	118.34(19)
C(8)-N-C(9)-C(11)	-127.74(19)
C(8)-N-C(9)-C(10)	110.4(2)
N-C(9)-C(10)-S	-62.63(19)
C(11)-C(9)-C(10)-S	175.23(12)
C(1)-S-C(10)-C(9)	105.90(14)
N-C(9)-C(11)-O(4)	-124.5(2)
C(10)-C(9)-C(11)-O(4)	-0.3(3)
N-C(9)-C(11)-O(3)	56.5(2)
C(10)-C(9)-C(11)-O(3)	-179.34(16)
O(4)-C(11)-O(3)-C(12)	3.4(3)
C(9)-C(11)-O(3)-C(12)	-177.54(16)

Table 7. Torsion angles [deg] for **4.40**. Symmetry transformations used to generate equivalent atoms.

Crystallographic data for twelve membered-serine ring 4.31

Empirical formula	C ₁₂ H ₁₇ N O ₅
Formula weight	255.27
Temperature	93(2) K
Wavelength	0.71073 Å
Crystal system, space group	Orthorhombic, P2(1)2(1)2(1)
Unit cell dimensions	a = 8.1341(18) Å alpha = 90 deg. b = 9.049(2) Å beta = 90 deg. c = 16.931(4) Å gamma = 90 deg.
Volume	1246.3(5) Å ³
Z, Calculated density	4, 1.360 Mg/m ³
Absorption coefficient	0.106 mm ⁻¹
F(000)	544
Crystal size	0.75 x 0.44 x 0.14 mm
Theta range for data collection	2.41 to 24.99 deg.
Limiting indices	-9<= <i>h</i> <=9, -10<= <i>k</i> <=9, -20<= <i>l</i> <=15
Reflections collected / unique	5297 / 2186 [R(int) = 0.0208]
Completeness to theta = 25.00	99.5 %
Absorption correction	Semi-empirical from equivalents
Max. and min. transmission	0.99 and 0.88055
Refinement method	Full-matrix least-squares on F ²
Data / restraints / parameters	2186 / 0 / 164
Goodness-of-fit on F ²	1.111
Final R indices [I>2sigma(I)]	R ₁ = 0.0256, wR ₂ = 0.0626
R indices (all data)	R ₁ = 0.0285, wR ₂ = 0.0637
Absolute structure parameter	-0.2(8)
Largest diff. peak and hole	0.114 and -0.193 e.Å ⁻³

Table 8. Crystal data and structure refinement for 4.31.

	x	y	z	U(eq)
C(1)	9083(2)	-551(1)	1860(1)	19(1)
C(2)	10597(2)	-211(2)	2318(1)	21(1)
C(3)	10343(2)	-542(2)	3187(1)	23(1)
C(4)	9085(2)	459(2)	3529(1)	22(1)
C(5)	7601(2)	57(2)	3731(1)	21(1)
C(6)	6274(2)	1068(2)	3991(1)	21(1)
C(7)	4855(2)	1095(2)	3403(1)	17(1)
C(8)	5404(2)	1742(1)	2636(1)	14(1)
C(9)	5865(2)	1332(1)	1248(1)	17(1)
C(10)	7237(2)	399(2)	911(1)	19(1)
C(11)	4466(2)	1331(1)	667(1)	18(1)
C(12)	2145(2)	52(2)	239(1)	29(1)
N	5371(1)	845(1)	2015(1)	15(1)
O(1)	8330(1)	-1677(1)	1893(1)	27(1)
O(2)	5872(1)	3020(1)	2593(1)	18(1)
O(3)	3507(1)	170(1)	768(1)	24(1)
O(4)	4277(1)	2232(1)	167(1)	29(1)
O(5)	8664(1)	576(1)	1388(1)	20(1)

Table 9. Atomic coordinates ($\times 10^4$) and equivalent isotropic displacement parameters ($\text{\AA}^2 \times 10^3$) for **4.31**. U(eq) is defined as one third of the trace of the orthogonalized Uij tensor.

C(1)-O(1)	1.1899(17)	C(7)-H(7B)	0.9700
C(1)-O(5)	1.3407(16)	C(8)-O(2)	1.2199(16)
C(1)-C(2)	1.487(2)	C(8)-N	1.3290(17)
C(2)-C(3)	1.515(2)	C(9)-N	1.4285(17)
C(2)-H(2A)	0.9700	C(9)-C(11)	1.5043(19)
C(2)-H(2B)	0.9700	C(9)-C(10)	1.5112(18)
C(3)-C(4)	1.484(2)	C(9)-H(9)	0.9800
C(3)-H(3A)	0.9700	C(10)-O(5)	1.4223(16)
C(3)-H(3B)	0.9700	C(10)-H(10A)	0.9700
C(4)-C(5)	1.307(2)	C(10)-H(10B)	0.9700
C(4)-H(4)	0.9300	C(11)-O(4)	1.1853(16)
C(5)-C(6)	1.481(2)	C(11)-O(3)	1.3193(17)
C(5)-H(5)	0.9300	C(12)-O(3)	1.4289(16)
C(6)-C(7)	1.5247(18)	C(12)-H(12A)	0.9600
C(6)-H(6A)	0.9700	C(12)-H(12B)	0.9600
C(6)-H(6B)	0.9700	C(12)-H(12C)	0.9600
C(7)-C(8)	1.4918(18)	N-H	0.8600
C(7)-H(7A)	0.9700		

Table 10. Bond lengths [Å] for **4.31**. Symmetry transformations used to generate equivalent atoms.

O(1)-C(1)-O(5)	123.28(13)	C(6)-C(7)-H(7B)	109.6
O(1)-C(1)-C(2)	125.39(12)	H(7A)-C(7)-H(7B)	108.1
O(5)-C(1)-C(2)	111.33(11)	O(2)-C(8)-N	122.50(12)
C(1)-C(2)-C(3)	110.58(12)	O(2)-C(8)-C(7)	121.17(11)
C(1)-C(2)-H(2A)	109.5	N-C(8)-C(7)	116.33(11)
C(3)-C(2)-H(2A)	109.5	N-C(9)-C(11)	112.43(11)
C(1)-C(2)-H(2B)	109.5	N-C(9)-C(10)	112.25(11)
C(3)-C(2)-H(2B)	109.5	C(11)-C(9)-C(10)	108.13(11)
H(2A)-C(2)-H(2B)	108.1	N-C(9)-H(9)	107.9
C(4)-C(3)-C(2)	110.65(12)	C(11)-C(9)-H(9)	107.9
C(4)-C(3)-H(3A)	109.5	C(10)-C(9)-H(9)	107.9
C(2)-C(3)-H(3A)	109.5	O(5)-C(10)-C(9)	109.02(11)
C(4)-C(3)-H(3B)	109.5	O(5)-C(10)-H(10A)	109.9
C(2)-C(3)-H(3B)	109.5	C(9)-C(10)-H(10A)	109.9
H(3A)-C(3)-H(3B)	108.1	O(5)-C(10)-H(10B)	109.9
C(5)-C(4)-C(3)	124.72(14)	C(9)-C(10)-H(10B)	109.9
C(5)-C(4)-H(4)	117.6	H(10A)-C(10)-H(10B)	108.3
C(3)-C(4)-H(4)	117.6	O(4)-C(11)-O(3)	124.29(13)
C(4)-C(5)-C(6)	125.37(14)	O(4)-C(11)-C(9)	124.41(12)
C(4)-C(5)-H(5)	117.3	O(3)-C(11)-C(9)	111.27(11)
C(6)-C(5)-H(5)	117.3	O(3)-C(12)-H(12A)	109.5
C(5)-C(6)-C(7)	111.53(12)	O(3)-C(12)-H(12B)	109.5
C(5)-C(6)-H(6A)	109.3	H(12A)-C(12)-H(12B)	109.5
C(7)-C(6)-H(6A)	109.3	O(3)-C(12)-H(12C)	109.5
C(5)-C(6)-H(6B)	109.3	H(12A)-C(12)-H(12C)	109.5
C(7)-C(6)-H(6B)	109.3	H(12B)-C(12)-H(12C)	109.5
H(6A)-C(6)-H(6B)	108.0	C(8)-N-C(9)	121.72(11)
C(8)-C(7)-C(6)	110.38(11)	C(8)-N-H	119.1
C(8)-C(7)-H(7A)	109.6	C(9)-N-H	119.1
C(6)-C(7)-H(7A)	109.6	C(11)-O(3)-C(12)	115.90(11)
C(8)-C(7)-H(7B)	109.6	C(1)-O(5)-C(10)	117.40(11)

Table 11. Bond angles [deg] for **4.31**.

	U11	U22	U33	U23	U13	U12
C(1)	18(1)	18(1)	20(1)	-1(1)	3(1)	4(1)
C(2)	15(1)	24(1)	25(1)	0(1)	1(1)	2(1)
C(3)	18(1)	28(1)	24(1)	1(1)	-3(1)	2(1)
C(4)	22(1)	25(1)	20(1)	0(1)	-2(1)	0(1)
C(5)	22(1)	21(1)	19(1)	6(1)	-2(1)	2(1)
C(6)	21(1)	26(1)	16(1)	3(1)	0(1)	0(1)
C(7)	17(1)	18(1)	17(1)	-1(1)	3(1)	0(1)
C(8)	11(1)	15(1)	18(1)	1(1)	0(1)	3(1)
C(9)	19(1)	15(1)	16(1)	2(1)	2(1)	-1(1)
C(10)	17(1)	23(1)	16(1)	0(1)	-1(1)	0(1)
C(11)	18(1)	16(1)	18(1)	0(1)	4(1)	4(1)
C(12)	22(1)	42(1)	23(1)	4(1)	-8(1)	-3(1)
N	19(1)	12(1)	15(1)	1(1)	1(1)	-2(1)
O(1)	25(1)	20(1)	37(1)	3(1)	-8(1)	-1(1)
O(2)	21(1)	14(1)	20(1)	-2(1)	1(1)	0(1)
O(3)	22(1)	27(1)	22(1)	6(1)	-8(1)	-5(1)
O(4)	28(1)	31(1)	27(1)	14(1)	-4(1)	1(1)
O(5)	17(1)	21(1)	20(1)	3(1)	0(1)	-2(1)

Table 12. Anisotropic displacement parameters ($\text{\AA}^2 \times 10^3$) for **4.31**. The anisotropic displacement factor exponent takes the form: $-2 \pi^2 [h^2 a^{*2} U_{11} + \dots + 2 h k a^* b^* U_{12}]$.

	x	y	z	U(eq)
H(2A)	10877	824	2252	26
H(2B)	11504	-798	2118	26
H(3A)	9992	-1559	3249	28
H(3B)	11373	-417	3467	28
H(4)	9374	1443	3604	27
H(5)	7357	-946	3710	25
H(6A)	6715	2058	4049	25
H(6B)	5869	752	4503	25
H(7A)	4457	97	3317	21
H(7B)	3959	1678	3616	21
H(9)	6268	2348	1296	20
H(10A)	6911	-631	903	22
H(10B)	7471	704	374	22
H(12A)	1455	907	292	44
H(12B)	1523	-820	363	44
H(12C)	2542	-12	-294	44
H	5043	-51	2076	18

Table 13. Hydrogen coordinates ($\times 10^4$) and isotropic displacement parameters ($\text{\AA}^2 \times 10^3$) for **4.31**.

O(1)-C(1)-C(2)-C(3)	-50.21(18)
O(5)-C(1)-C(2)-C(3)	130.54(12)
C(1)-C(2)-C(3)-C(4)	-65.36(15)
C(2)-C(3)-C(4)-C(5)	107.81(16)
C(3)-C(4)-C(5)-C(6)	-172.97(13)
C(4)-C(5)-C(6)-C(7)	114.20(15)
C(5)-C(6)-C(7)-C(8)	-65.47(14)
C(6)-C(7)-C(8)-O(2)	-63.16(16)
C(6)-C(7)-C(8)-N	116.02(12)
N-C(9)-C(10)-O(5)	-64.66(14)
C(11)-C(9)-C(10)-O(5)	170.74(10)
N-C(9)-C(11)-O(4)	142.93(13)
C(10)-C(9)-C(11)-O(4)	-92.58(15)
N-C(9)-C(11)-O(3)	-39.13(15)
C(10)-C(9)-C(11)-O(3)	85.37(13)
O(2)-C(8)-N-C(9)	-0.85(19)
C(7)-C(8)-N-C(9)	179.98(12)
C(11)-C(9)-N-C(8)	-115.10(13)
C(10)-C(9)-N-C(8)	122.71(12)
O(4)-C(11)-O(3)-C(12)	-1.4(2)
C(9)-C(11)-O(3)-C(12)	-179.35(11)
O(1)-C(1)-O(5)-C(10)	0.48(19)
C(2)-C(1)-O(5)-C(10)	179.74(11)
C(9)-C(10)-O(5)-C(1)	106.45(13)

Table 14. Torsion angles [deg] for **4.31**. Symmetry transformations used to generate equivalent atoms.